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THE EFFECT OF MUSCULAR WORK, DIET AND HEMOLYSIS ON THE SERUM PROTEINS

TOGETHER WITH COMMENT ON THE TECHNIC AND CLINICAL USEFULNESS OF ROBERTSON'S MICROREFRACTOMETRIC METHOD *

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MUSCULAR WORK

Böhme[1] has shown that muscular work, even of the simplest sort, increases the serum concentration. Schwenker[2] found this concentration returned to a constant normal level after the patient had reclined quietly for twenty minutes. His explanation of the increase was that during work, blood pressure rises, which forces protein-poor fluids out of the blood vessels into the surrounding tissues. Reiss,[3] on the other hand, thought that during activity muscles take up considerable water, giving it up during rest.

Our investigations on this effect of work on serum proteins in normal men show a very decided increase in the total proteins, which increase occurs more in the albumin than in the globulin fraction. The nonproteins increase very slightly. The work done was on a bicycle ergometer to a point where the subjects were near exhaustion, except in Case 3, in which the increase in proteins due to merely walking around the laboratory is shown. On two cases, the degree of acidosis due to work was estimated by Van Slyke's recent method. Thus, these results confirm former work with the refractometer on this subject and show the manner in which this increase is split between the serum albumin and globulin as well as the effect of work on the nonproteins.

FOOD AND FLUIDS

Böhme[1] and Reiss[3] have shown, by the refractometer, that the concentration of serum is only slightly changed after a large meal, this change following no rule, being either an increase or decrease. We

* Submitted for publication Aug. 29, 1916.

* From the Medical Service and Chemical Laboratory of the Massachusetts General Hospital, Boston.

1. Böhme: Deutsch. Arch. f. klin. Med., 1911, **103,** 522.
2. Schwenker: Inaugural Diss., Wurzburg, 1905.
3. Reiss: Ergebn. d. inn. Med. u. Kinderh., 1913, **10,** 531.

have used Robertson's microrefractometric method[4] to reinvestigate this problem, taking the serum from one to one and one-half hours after the test meal, during which period Böhme showed slight changes in the serum concentration. The test meal was high in carbohydrates and protein. Little fat was given, so that a lipemia would not interfere with the refractometric readings. Our results show either a slight increase or slight decrease in the serum proteins, which agrees with former findings. Serum albumin changed more than globulin, except in Case 5. Nonproteins were not changed except for 0.1 per cent. in Case 1.

The effect of high protein diets given over a number of days on serum proteins was investigated refractometrically by Chajes,[5] who obtained slight increases in some cases with stationary values in others.

TABLE 1.—Effect of Work on Serum Proteins in Normal Men

Case No.	Serum Proteins Before Work*				Work Done in Kilogrammeters	Serum Proteins After Work				Increase in Proteins Due to Work				Volume Percentage of CO_2 Bound by Blood Plasma after Work†
	Albumin	Globulin	Total Protein	Nonprotein		Albumin	Globulin	Total Protein	Nonprotein	Albumin	Globulin	Total Protein	Nonprotein	
1	5.9	1.6	7.5	1.3	16,995	6.7	1.9	8.6	1.4	0.8	0.3	1.1	0.1	....
2	5	1.7	6.7	1.3	Slight exercise with walking	5.3	1.9	7.2	1.3	0.3	0.2	0.5	0	....
3	5	1.7	6.7	1.3	11,280	6.5	2.1	8.6	1.3	1.5	0.4	1.9	0	41.9
4	6	1.5	7.5	1.3	14,797	7	2.1	9.1	1.35	1	0.6	1.6	0.05	48

* Serums were taken from patients who had been lying down for at least twenty minutes.
† Van Slyke's method.

Our results show that after four days of high protein diet there is on the average a slight increase in the total proteins, and we found no definite division of this increase between the albumin and globulin fractions. The nonproteins were slightly increased in three cases, decreased in one case, remaining stationary in two cases.

As a result of this work on the effect of diet on serum proteins, it seems that an immediate effect is practically wanting, while a long continuance of high protein diets slightly increases the serum proteins.

In this connection it is worth while noting that Engel and Scharl[6] and Reiss[3] found no definite sort of variation in normals or in heart or kidney cases at any time after drinking large amounts of water or

4. Robertson: Jour. Biol. Chem., 1915, **22**, 233.
5. Chajes: Therap. d. Gegenw., 1904, **45**, 442.
6. Engel and Scharl: Ztschr. f. klin. Med., 1906, **60**, 225.

milk. Markus[7] found a slight increase in the serum concentration of a chronic nephritic as a result of drinking water. On the other hand, the refractometric work of Benczur[8] and others has shown that the concentration of serum does decrease when extra salt is taken into the system, this decrease disappearing soon in normal people, but persisting for long periods when kidney damage is present.

VARIATIONS ASSOCIATED WITH NONSEPTIC HEALING

We have noticed that the percentage of globulin has been rather high when fractures or postoperative hernial incisions were healing. In only one case, though, was this percentage above normal limits. These percentages in healing fractures were 39, 31, 27, 30, 27.5 and 30, while in healing herniotomy incisions they were 31, 29, 29. These patients were all confined to bed and were running no fever, and no septic foci were present.

TABLE 2.—IMMEDIATE EFFECT ON SERUM PROTEINS BY FOOD

Case No.	Serum Proteins Three Hours After Breakfast				Caloric Intake of Test Meal				Serum Proteins 1 or 1½ Hours after Test Meal				Variations in Serum Proteins Due to Food			
	Albumin	Globulin	Total Protein	Nonprotein	Protein	Fat	Carbohydrates	Total Calories	Albumin	Globulin	Total Protein	Nonprotein	Albumin	Globulin	Total Protein	Nonprotein
1	4.8	2	6.8	1.3	135	39	530	754	4.9	2	6.9	1.2	0.1	0	0.1	—0.1
2	5.6	1.8	7.4	1.2	126	28	449	606	5.2	1.7	6.9	1.2	—0.4	—0.1	—0.5	0
3	4.6	1.9	6.5	1.25	180	38	510	728	4.5	1.9	6.4	1.25	—0.1	0	—0.1	0
4	4.6	2.1	6.7	1.2	135	39	464	638	4.8	2.2	7	1.2	0.2	0.1	0.3	0
5	6.5	1.7	8.2	1.3	*	*	*	*	6.4	1.4	7.8	1.3	—0.1	—0.3	—0.4	0

* Meal similar to those above.

Though these values are within normal limits, except in one case, the repeated finding of results in the upper limits of the normal variation is probably of significance. It seems possible that this elevation above the normal average of about 24 per cent. might be due to the effect of toxic split proteins liberated in the healing processes. It is assumed that such toxic substances increase the globulin, in the same way that nonspecific protease or vaccines produce leukocytosis and undoubtedly raise the globulin, as shown in recent articles.[9]

CHANGES IN SERUM PROTEINS DUE TO KEEPING SERUMS ON ICE FROM TWENTY-FOUR TO FORTY-EIGHT HOURS

Table 4 demonstrates that if serums are kept on ice, they can be examined at any time within forty-eight hours after the blood-letting

7. Markus: Berl. klin. Wchnschr., 1907, **44**, 506, 537.
8. Benczur: Ztschr. f. klin. Med., 1909, **62**, 164.
9. Jobling and Petersen: Jour. Am. Med. Assn., 1916, **66**, 1753.

without fear of changes in the serum proteins. In Cases 10 and 11 the serum was left in contact with the clot, which made no important difference in the results. The variations present are nearly within the limits of error of this technic.[10]

HEMOLYSIS

The effect of hemolysis on serum proteins is shown in Table 5. In Case 2 it was hemolyzed as much as possible by repeated freezing and thawing without preventing its reading with a sodium flame. The increase in total protein is considerable, the increase in the albumin being either greater than or equal to that in the globulin. There is also a slight increase in the nonproteins. The gradual decrease in the refractometric reading as hemolysis of Serum 2 was increased is given in this place:

Degree of Hemolysis	Refractometric Reading
No hemolysis	64° 3′
Slight hemolysis	64° 1′
Moderate hemolysis	63° 55′
Limit of hemolysis permitting refractometric reading	63° 45′

The slight amounts of hemolysis often present in serums only make a difference of a few seconds in the reading and thus practically no difference in the estimated serum proteins. If a slightly hemolyzed serum is perfectly easy to read with a sodium flame, the amount of increase in the serum proteins over the normal may be ignored except for the most accurate work. In our investigations we have used no serums that were hemolyzed except in a very few instances and then the hemolysis was always very slight.

TECHNIC

Throughout all our work on serum proteins the refractometric method as described by Robertson[4] has been followed. The comment on this technic here given may aid those who wish to use the method in further investigations.

As mentioned in a former article,[11] the syringe into which blood is drawn must be dry and clean so as not to dilute or change the blood serum. That article also shows the necessity of drawing blood from the vein within one minute after the tourniquet is applied so as not to increase the serum concentration by venous stasis.

The results of the present article show that in order to exclude the increase of serum proteins caused by any muscular activity, all patients should have been in a reclining position for at least twenty minutes

10. The limits of error due to technic in Robertson's microrefractometric method are about ± 0.2 per cent. albumin, ± 0.15 per cent. globulin, and ± 0.1 per cent. nonproteins.

11. Rowe: Jour. Lab. and Clin. Med., 1916, **1**, 485.

before blood is drawn. Though an immediate effect of food on serum proteins has been found to be practically wanting, in order to obviate lipemia, which makes the refractometric reading difficult, the taking of blood three hours after the last meal is desirable. Serums which are more than slightly hemolyzed should not be used. If inconvenient to estimate serum proteins immediately, the serum may be kept on ice and examined within the following forty-eight hours.

A few points on the manipulation of the serum follow. The writer has found that his automatic pipet,[12] recently described, measures fluids better than can be done by hand, saving much time and insuring accuracy after its simple operation has been learned. In estimating nonproteins 0.5 c.c. of serum has been found sufficient for each test. To assure accuracy, two controls for globulin estimations and one control for nonprotein estimations are advised, thus making the setting

TABLE 3.—EFFECT OF HIGH PROTEIN DIET ON SERUM PROTEINS

Case No.	Diagnosis	Low Protein Diet for Four Days; Serum Proteins				Average Daily Urinary N Output in Grams*	High Protein Diet for Four Days; Serum Proteins				Average Daily Urinary N Output in Grams*	Variations in Serum Proteins Due to High Protein Diet			
		Albumin	Globulin	Total Protein	Nonprotein		Albumin	Globulin	Total Protein	Nonprotein		Albumin	Globulin	Total Protein	Nonprotein
1	Fractured femur	4.5	2.9	7.4	1.2	6.6	4.7	2.7	7.4	1.3	15.6	0.2	—0.2	0	0.1
2	Fractured femur	5.2	1.9	7.1	1.3	....	5.5	2.3	7.8	1.3	....	0.3	0.4	0.7	0
3	Septic knee	3.8	3.4	7.2	1.5	6.66	4.3	3.4	7.7	1.3	12.3	0.5	0	0.5	—0.2
4	Fractured tibia	5.8	2.2	8	1.1	5.95	5.6	2.3	7.9	1.25	20.16	—0.2	0.1	—0.1	0.15
5	Fractured hip	5.1	2.2	7.3	1.2	5.79	5.1	2.3	7.4	1.3	11.7	0	0.1	0.1	0.1
6	Septic compound fract.	4.4	2.7	7.1	1.3	4.4	4.7	2.6	7.3	1.3	9.27	0.3	—0.1	0.2	0

* Estimation of urinary nitrogen was kindly furnished by Dr. W. Dennis.

up of five separate tubes for each serum. It has been found important to keep all tubes capped at all times after the fluid has been introduced, to prevent evaporation, this closure being done as advised by Robertson. Instead of platinum or silver wire, a rather thick chromium wire cut into lengths of about 1 or 1.5 cm. have been very satisfactory to bring about mixture of serum, sulphate solution and acetic acid solution. The separation of the coagulum resulting from boiling the nonprotein tubes is accomplished by means of a slender glass rod rather than by a platinum wire. A piece of chromium wire would serve the same purpose. In order to prevent bumping during the boiling of these nonprotein tubes, a double piece of wire gauze cut to fit the bottom of the beaker is better than cotton. The refractometric

12. Rowe: Jour. Lab. and Clin. Med., 1916, **1**, 439.

readings have been made in a fairly dark room where, by means of a window and door, the temperature was kept nearly constant during the readings.

That this temperature regulation serves the same purpose as does the complicated Pulfrich temperature regulating apparatus is shown by the following results obtained by examining a serum by the University of California instrument, which we used, and by a refractometer

TABLE 4.—Changes in Serum Proteins Caused by Keeping Serum on Ice

Case No.	Serum Proteins within 4 Hours after Blood-Drawing					Time Serum Stood on Ice between Examinations, Hours	Variations in Serum Proteins after Serum Stood on Ice				
	Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin		Albumin	Globulin	Total Protein	Nonprotein	Per Cent. of Globulin
1	4.2	2.3	6.5	1.3	36	48	0	−0.1	−0.1	0	−2
2	4.8	3.1	7.9	1.2	40	48	0.1	−0.3	−0.2	0.1	−4
3	5.1	2.9	8	1.6	36	24	0	0	0	0	0
4	5.7	2	7.7	1.1	26	24	0.3	−0.1	0.2	−0.1	−1
5	5.8	2.9	8.7	1.3	33	24	−0.3	0.2	−0.1	0.1	3
6	4.3	2.1	6.4	1.4	33	24	0.2	−0.1	0.1	0	−2
7	4.9	2.2	7.1	1.1	31	24	−0.2	0	−0.2	0.2	1
8	4.8	2.1	6.9	1.3	30	24	−0.1	0	−0.1	0	1
9	5.9	3	8.9	1.3	34	24	−0.1	0.1	0	0.1	1
10	4.4	2.2	6.6	1.5	33	24	0.1	−0.3	−0.2	0	−3
11	4.5	2	6.5	1.4	31	24	0.2	−0.2	0	0	−3

belonging to the Institute of Technology, which had the temperature regulating device:

	Univ. of Calif., Per Cent.	Technology, Per Cent.
Albumin	5.2	5.24
Globulin	2.07	2.09
Total protein	7.27	7.33
Nonprotein	1.3	1.3

The agreement of these results shows the accuracy of the instrument used in our investigations and also the possibility of obtaining accurate results by keeping the room temperature constant. In order that the fluids to be examined might be at room temperature they were left in that room one-half hour before readings were taken. If a 15 watt electric light bulb is suspended just above and in front of the refractometer, the readings may be made without turning the light on and off.

POSSIBLE CLINICAL USE OF THIS METHOD

For clinical use this method of investigation of blood serum is practical, especially if in diseases in which no definite increase in nonproteins has been found, our average value of 0.00198 for these nonproteins is used. As stated in a previous article,[13] if this fixed value is used, the serum examination is greatly shortened and an unreasonable error is not introduced. In severe nephritis, especially where uremia[14] is present, it is necessary to make determinations of nonproteins in each serum to obtain accurate results.

We have shown that during certain diseased conditions, especially generalized infections and cardiorenal diseases, that the total proteins may be decreased and the percentage of globulin in the total proteins increased, and that recovery is accompanied by a return of these values to normal levels. A guide of some worth as to prognosis and treatment seems to reside in the following of these values during the course of the disease, especially in chronic nephritis with edema. Diabetes has been found to be accompanied by normal percentages of

TABLE 5.—EFFECT OF HEMOLYSIS ON SERUM PROTEINS

Case No.	Serum Proteins before Hemolysis					Increase in Serum Proteins Due to Hemolysis				
	Albumin	Globulin	Total Pro-	Nonprotein	Per Cent. of Globulin	Albumin	Globulin	Total Pro-	Nonprotein	Per Cent. of Globulin
1	5.7	2	7.7	1.3	26	0.2	0.2	0.4	0.1	1
2	5.9	1.6	7.5	1.3	21	0.5	0.2	0.7	0.05	1

globulin, except for an increase when a complicating infection is present. Other points of clinical interest are mentioned throughout our other articles.

We have commented before on the inaccuracy of estimating total proteins by the table of Reiss.[15] This error would be slightly less if 0.00198 were substituted for his value of 0.00277 for nonproteins. Determination of total proteins, though, by this method should be used only when a rough idea of the serum concentration is desired, in such cases as Reiss mentions in his last article, for instance, in which a daily comparison of serum concentration with body weight would be of use.

SUMMARY

Muscular activity, even of the simplest sort, increases total serum proteins, this increase occurring more in the albumin than the globulin fraction.

13. Rowe: THE ARCHIVES INT. MED., 1916, **18,** 455.
14. Rowe: THE ARCHIVES INT. MED., 1917, **19,** 354.
15. Reiss: Deutsch. Arch. f. klin. Med., 1915, cvii, 175.

An immediate effect of diet on serum proteins is practically wanting, while a high protein diet continued for several days produces a slight increase in the serum proteins.

The percentage of globulin seems to be slightly increased in cases in which healing of fractures or operative incisions is occurring and in which no signs of infection are present.

Serum proteins undergo no marked changes after the serum has been kept on ice for forty-eight hours.

Hemolysis, when excessive, definitely increases serum concentration, while slight hemolysis produces only a very slight increase.

A few suggestions about the technic and clinical use of Robertson's microrefractometric method are given.

THE CAUSES OF VARIATION IN THE CONCENTRATION OF UREA IN THE BLOOD OF YOUNG HEALTHY ADULTS *

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SAN FRANCISCO

It is known that there may be a variation of more than 100 per cent. in the blood urea concentration of any group of normal subjects. It is not known why this considerable degree of variation should exist. From the available data it is possible to suppose that each person has his own characteristic blood urea concentration, which varies only within narrow limits, although the variation between different subjects is so large. Or it may be that the blood urea concentration in each person varies widely in accordance with one or other of the many constantly changing conditions of the body.

One cause of variation in the concentration of some constituents of the blood of different persons is a variable food intake. And before any study of the effect of physiologic variables on the blood urea concentration can be carried out or any attempt made to draw any but the broadest line between normal and abnormal, it is necessary to determine the range of variation in subjects in whom this possible cause of variation has been removed by the imposition of a qualitatively and quantitatively constant diet. Yet we do not so much as know whether food alterations can influence the blood urea concentration in normal subjects. It is only in the most recent work that even inadequate attention has been paid to the possibility of the effect of dietary factors. Bang[1] carried out determinations of nonprotein nitrogen, urea nitrogen and amino-acid nitrogen on eight students while they were on a diet which was more or less qualitatively the same, though it is not stated that any quantitative restrictions were imposed. His methods are analogous to his micromethod for sugar estimation in the blood, in that only one or two drops of blood are used. He found a variation of from 0.006 to 0.022 per cent. urea nitrogen before breakfast. He states that under ordinary conditions dietary changes have no effect on the blood urea concentration of normal persons. Schwartz and McGill[2] found a variation of from 0.0108 to 0.0156 per cent. urea nitrogen in four normal subjects after

* Submitted for publication Aug. 15, 1916.

* From the Laboratory of the Medical Division of the Leland Stanford Junior University School of Medicine, San Francisco.

1. Bang, I.: Biochem. Ztschr., 1915, **62**, 104.
2. Schwartz, H., and McGill, C.: THE ARCHIVES INT. MED., 1916, **17**, 42.

TABLE 1.—The Effect of Variation in Diet on the Urea Concentration of the Blood in Normal Individuals

Date	Diet			Urea in Urine 24 Hours, Gm.	Urea in Blood, Grams per 100 C.c.
	Protein, Gm.	Carbohydrate, Gm.	Fat, Gm.		
Subject Add.					
4/17-18	12	325	45	16.02	
4/18-19	12	333	45	10.92	
4/19/20	12	350	45	9.2	
4/20-21	12	328	45	8.39	0.0225
4/21-22	12	450	45	8	0.027
4/22-23	12	390	45	8.25	
4/23-24	132	7	212	15.81	
4/24-25	145	6	218	28.29	
4/25-26	130	6	229	36.1	
4/26/27	148	12	284	36.09	0.0567
4/27-28	115	8	235	34.34	0.0489
4/28-29	110	6	225	32.7	0.0426
4/29-30	75	220	120	21.95	
4/30- 1	75	220	120	14.02	
5/ 1- 2	75	220	120	15.82	
Subject Mo.					
4/17-18	12	342	45	.....	
4/18-19	12	382	45	12.82	
4/19-20	12	390	45	10.14	
4/20-21	12	491	45	8.74	0.024
4/21-22	12	497	45	10.55	0.0252
4/21-23	12	440	45	6.25	0.0232
4/23-24	155	9	200	18.49	
4/24-25	132	7	249	22.6	
4/25-26	164	9	300	47.28	
4/26-27	192	6	279	41.95	0.0468
4/27-28	140	9	255	50.02	0.0369
4/28-29	215	8	279	40.45	0.0402
4/29-30	75	220	120	29.39	
4/30-31	75	220	120	16.81	
5/ 1- 2	75	220	120	13.15	
Subject Bo.					
5/ 2- 3	11	402	76	16.22	
5/ 3- 4	11	482	76	11.88	
5/ 4- 5	11	400	76	7.17	
5/ 5- 6	11	455	76	7.36	0.0168
5/ 6- 7	11	455	76	8.34	0.0169
5/ 7- 8	11	455	76	5.74	0.0228

TABLE 1.—THE EFFECT OF VARIATION IN DIET ON THE UREA CONCENTRATION OF THE BLOOD IN NORMAL INDIVIDUALS—(*Continued*)

Date	Diet			Urea in Urine 24 Hours, Gm.	Urea in Blood, Grams per 100 C.c.
	Protein, Gm	Carbohydrate, Gm	Fat, Gm.		
5/ 8- 9	161	4	238	25.08	
5/ 9-10	161	4	238	43.24	
5/10-11	161	4	238	40.35	
5/11-12	161	4	238	37.42	0.039
5/12/13	161	4	238	35.58	0.041
5/13-14	161	4	238	35.18	0.042
Subject H.					
5/ 2- 3	11	437	76	13.02	
5/ 3- 4	11	432	76	8.26	
5/ 4- 5	11	407	76	6.01	
5/ 5- 6	11	417	76	5.57	0.0147
5/ 6- 7	11	412	76	6.13	0.0125
5/ 7- 8	11	417	76	5.86	0.0228
5/ 8- 9	154	4	233	21.87	
5/ 9-10	161	4	258	44.35	
5/10-11	154	4	233	49.5	
5/11-12	154	4	233	42.23	0.0438
5/12-13	134	4	177	40.05	0.0357
5/13-14	154	4	233	44.24	0.0384
5/14-15	75	220	120	26.62	
5/15-16	75	220	120	16.55	
5/16-17	75	220	120	14.4	

seventeen hours' fasting. In fifteen normals two and a half hours after "a heavy protein meal" the variation was from 0.0108 to 0.0252 per cent. urea nitrogen. Gettler and Baker[3] have published a very valuable series of determinations of various blood constituents in thirty normal persons. They found a variation of from 0.01 to 0.026 per cent. urea nitrogen three hours after a standard breakfast.

It does not appear certain from these data that dietary changes have any effect on the urea concentration of the blood of normal subjects, although Folin, Denis and Seymour[4] have shown that in patients with high blood pressure an increase of protein in the diet leads to an increase in the urea concentration of the blood. This, however, might be and no doubt has been ascribed entirely to defective elimina-

3. Gettler and Baker: Jour. Biol. Chem., 1916, **25**, 211.

4. Folin, O., Denis, W., and Seymour, M.: THE ARCHIVES INT. MED., 1914, **13**, 224.

tion of urea by the kidneys. With kidneys of unimpaired efficiency, on the other hand, it might well be imagined that any marked alteration in the level of urea in the blood would be obviated by an automatic increase in the rate of urea excretion whenever the urea concentration of the blood began to rise. But this is not what happens. In healthy subjects as well as in those whose kidneys may be defective, an increased nitrogen consumption is accompanied by an increased urea content of the blood. This is demonstrated in the experiments detailed in Table 1.

It will be noted that there is a considerable increase in blood urea concentration[5] when the food is changed from a mainly carbohydrate to a protein-fat diet. Therefore, in order to determine what degree of variation may arise from other than food factors, it is necessary that all the subjects should be on the same diet.

The figures given in Table 2 were obtained at 10:30 a. m. on the third and sixth days of the same diet. On the fourth, fifth and sixth days of the diet 20 or 40 gm. of urea were taken at 8 a. m. The subjects were instructors and students between the ages of 20 and 35 years. The diet has been given in detail elsewhere,[6] as well as the method used for the estimation of urea (a slight modification of Van Slyke and Cullen's method).[7] Five c.c. of blood was the smallest amount used for each estimation. Duplicate estimations were made in the great majority of cases.

The chief interest of these figures seems to us to lie in their demonstration of the diversity of concentration which may exist in normal subjects in spite of uniformity of diet. This is particularly true of the third day of the diet, for after urea administration the range of variation becomes relatively narrower. But before urea was taken we have variations of from 0.0156 to 0.0438 per cent.

Eighty-two per cent. of our twenty-eight cases had a concentration of 0.02 per cent. Only three cases, Ad., We. and M., show a wide variation from the average. It will be noted that these three cases three days later after urea was taken still show an unusually high or low urea concentration. It would appear then that these differences are to be ascribed in part at least to individual peculiarities which continued to exist throughout the period of the test and are not due altogether to temporary and evanescent causes of variation. Now we have shown that the rate of excretion of administered urea

5. All the figures for blood urea concentration in this paper refer to grams of *urea* per hundred c.c. of blood and not to urea nitrogen. The values for urea nitrogen may be approximately obtained by dividing our results by 2.

6. Addis, T., and Watanabe, C. K.: Jour. Biol. Chem., 1916, **27**, 249. There was no egg given with the noonday meal, as is incorrectly stated in this paper.

7. Addis, T., and Watanabe, C. K.: Jour. Biol. Chem., 1916, **24**, 203.

TABLE 2.—CONCENTRATION OF UREA IN THE BLOOD OF NORMAL INDIVIDUALS ON A CONSTANT DIET ARRANGED IN ORDER OF DESCENDING MAGNITUDE

Blood Urea Concentration, Gm. per 100 C.c.					
Subject	Before Urea Administration, 3d Day 10-11 a. m.	Subject	After 20 Gm. Urea at 8 a. m., 6th Day 10-11 a. m.	Subject	After 40 Gm. Urea at 8 a. m., 6th Day 10-11 a. m.
Ad.	0.0438	Ad.	0.075	*Eh.	0.123
D.	0.0312	A.	0.069	*Sh.2 *K.2	0.1125 0.105
Bo.2 Sh.1	0.03 0.0292	Add.	0.0678	*Bo.2	0.096
G.	0.0288	Bo.1	0.0672	*T.	0.09
A.	0.0282	K.2	0.0672		
Add.	0.0276	K.1	0.066		
*Sh.2	0.027	D.	0.0651		
Bo.1	0.0261	J.	0.063		
Br.1	0.0252	Wa.	0.063		
Cha.	0.0252	C.	0.0624		
T.	0.0252	G.	0.0612		
J.	0.0252	S.1	0.0606		
Wa.	0.025	Br.1	0.06		
C.	0.0246	Boy	0.059		
*McC.	0.0246	C.	0.0558		
W.	0.0246	F.	0.0558		
K.1	0.0246	M. O.	0.0558 0.0546		
B.	0.024	B.	0.0516		
F.	0.024	We.	0.051		
S.1	0.024	W.	0.0492		
*K.2	0.0216				
*K.3	0.0216				
C.	0.021				
W.	0.021				
Ma.	0.021				
O.	0.0204				
We.	0.018				
M.	0.0156				

* Blood collected at 11 a. m. instead of 10:30 a. m. The inferior figures indicate repetitions of the diet by the same individual.

in normal persons is remarkably constant and that these three subjects were not exceptions to this rule.[6] The high blood urea concentration of Ad. is therefore certainly not due to a relative insufficiency of his kidneys, nor did the low concentrations in We. and M. arise because their kidneys were exceptionally efficient. We believe that the explanation is to be found in the fact that the level of protein catabolism

was high in the subject Ad. and low in We. and M. Although these subjects took the same amount of nitrogen, the urea excretion over the whole period of the diet, with the exception of the first day, was 142.9 gm. for Ad. and only 123.8 gm. for We. and 110.4 gm. for M. The high urea excretion of Ad. presumably arose from an increased conversion of amino-acids from his tissues and from the protein of his food into urea, whereas the low urea excretions of We. and M. may be accounted for by a decreased tissue catabolism and a high degree of utilization of food amino-acids for protein synthesis, with a consequent decrease in urea formation.

In explanation of this effect of increased protein catabolism in raising the blood urea concentration, it is not necessary to assume any peculiarity in the urea derived from tissue catabolism whereby it is more difficult to excrete and so tends to heap itself up in the blood. For, as is shown by our results after urea administration, exactly the same effect is produced by preformed urea taken by mouth. Urea, whether from the alimentary tract or from the tissues, will tend to raise the blood urea concentration whenever it enters the blood more rapidly than it leaves it through the kidneys. The kidneys are not so constituted as to be close regulators of the level of urea in the blood, in the manner, for instance, in which the respiratory center maintains an even hydrogen ion concentration. There is no necessity for any such fine adjustment, since the properties of urea are such that considerable fluctuations in concentration are immaterial to the body.

But we believe that alterations in blood concentration may occur also as a result of changes in the sensitiveness of the kidneys to the stimulation to an increased rate of urea excretion which is provided by rises in blood urea concentration. Under the constancy of the conditions imposed on the series of persons we have detailed above, this factor was probably of very minor importance, yet even there it seems probable that the rate of protein catabolism does not explain all the variation found, for there are instances in which there is no very close correspondence between the blood urea concentration and the rate of protein catabolism. But it is particularly under widely different conditions that this factor of varying states of kidney activity seems to become evident. Thus, in a case of diabetes, after forty-eight hours during which nothing was taken but whisky and water, we gave 20 gm. of nitrogen-free corn starch, which did not induce any glycosuria. Some hours later we estimated the blood urea concentration in the expectation that we would find an unusually low concentration, because the rate of protein metabolism, as estimated by the urinary excretion, was low, the carbohydrate had been utilized and all exogenous sources of urea cut off. Yet 0.063 per cent. was found. A month later, after the patient had been for some time on a

diet with a moderate amount of protein and almost no carbohydrate we found a concentration of only 0.0462 per cent. a few hours after a breakfast of two eggs and some bacon. The rate of urea excretion in the urine on the first occasion was only 0.48 gm. per hour, whereas at the second examination, in spite of the lower blood concentration, it was 1.43 gm. per hour. This is only the most marked of a series of observations which have led us to conclude that the level of urea in the blood may increase, not only because urea comes in too quickly, but also because under certain conditions it may leave the blood too slowly. In other words, there is no necessarily fixed relationship between the concentration of urea in the blood and the rate of urea excretion, since other factors than urea play a part in determining the rate of excretion.

The wide range of variation in these results is also of interest in connection with the significance to be attached to estimations of blood urea concentration in patients. On a diet such as ours, a conservative estimate would call abnormal only concentrations which did not fall within from 0.01 to 0.05 per cent [illegible] when, as must commonly be the case, the patient has not been [illegible]ected to any dietetic preparation, figures beyond these limits may still possibly be normal, since under variable food conditions the normal range of variation will be widened.

The simplest way to attain some degree of constancy is to take the blood in the morning before breakfast. It is, however, not to be expected that in this manner any such uniformity can be attained as is possible when a constant diet is taken for several days. When a person passes from an unrestricted diet containing a moderate quantity of nitrogen to a fixed diet containing slightly less nitrogenous food, it requires on an average a full twenty-four hours for an equilibrium in the excretion of urea to be established. But when the nitrogen in the food taken before the diet exceeds at all markedly the amount in the fixed diet, the excretion of the excess urea is apparent for as long as forty-eight hours. Constancy as regards diet, therefore, is not to be attained by abstention from food for the twelve to seventeen hours, which represents the time between the evening meal on the previous day and the taking of the blood before breakfast. Yet in clinical work this is not infrequently the highest degree of uniformity in conditions which can be obtained.

We have thought it well, therefore, to collect all our observations on normal subjects when the blood was taken before food on a day following unrestricted diet. Some of these subjects had taken water, but as we shall show later, this does not demonstrably alter the blood urea concentration. In order that the extent of the error inherent in the method of estimation may be appreciated, we have given both the results obtained in all those cases in which double estimations were

TABLE 3.—Concentration of Urea in the Blood of Normal Fasting Adults

ARRANGED IN ORDER OF ACENDING MAGNITUDE; DUPLICATE ESTIMATIONS

No.	Subject	Blood Concentration in 100 C.c.		No.	Subject	Blood Concentration in 100 C.c.	
1	V.	0.0225		40	Jo.	0.0336	0.0336
2	Add.	0.0246	0.0246	41	Add.	0.0337	
3	Add.	0.0264	0.0264	42	So.	0.0342	0.033
4	Add.	0.0264	0.0264	43	Add.	0.0342	0.033
5	Add.	0.027	0.0258	44	Ka.	0.0337	
6	Mon.	0.027	0.0264	45	Add.	0.0354	0.0345
7	V.	0.027		46	Add.	0.0354	0.0354
8	Add.	0.0276	0.0276	47	Add.	0.036	0.0354
9	Mon.	0.0282	0.027	48	Ka.	0.036	
10	Mon	0.0282		49	Add.	0.036	0.036
11	Kl.	0.0282	0.0282	50	Mon.	0.036	0.036
12	Mon.	0.0288	0.0276	51	Wl.	0.0366	0.036
13	Cal.	0.029		52	So.	0.0366	0.036
14	Sh.	0.0292		53	Add.	0.0367	
15	Cal.	0.0292		54	Add.	0.0372	0.0364
16	Add.	0.0294	0.0294	55	Sha.	0.0379	
17	Mon.	0.03	0.0288	56	So.	0.0382	
18	Wll.	0.03	0.0294	57	Cal.	0.0384	0.0372
19	Add.	0.03	0.0294	58	Ja.	0.0384	0.0378
20	Add.	0.03		59	Mo.	0.0384	0.0381
21	Add.	0.03	0.03	60	Ch.	0.039	0.039
22	Add.	0.03	0.03	61	Ja.	0.039	
23	Add.	0.03		62	Ja.	0.0397	
24	Ka.	0.0307		63	Ja.	0.0396	0.0392
25	Add.	0.0312	0.0306	64	Add.	0.0402	0.039
26	Add.	0.0312	0.0312	65	Mo.	0.0402	0.0396
27	Add.	0.0312	0.0312-	66	Ka.	0.0402	0.039
28	Mon.	0.0315	0.0315	67	Mo.	0.0402	
29	Add.	0.0315		68	Jo.	0.0405	
30	Add.	0.0318	0.0306	69	Add.	0.0408	0.039
31	Mon.	0.0318	0.0312	70	F.	0.0408	
32	Add.	0.0322		71	Add.	0.0414	0.0408
33	Add.	0.0322		72	Add.	0.0414	0.0414
34	Mon.	0.0324	0.0312	73	Add.	0.0414	0.0408
35	Boy	0.0324	0.0318	74	Pr.	0.042	0.0414
36	Sh.	0.033		75	Jo.	0.0426	0.042
37	Add.	0.0333	0.033	76	Add.	0.0426	0.0426
38	Add.	0.0336	0.0324	77	Add.	0.0426	0.0426
39	Ka.	0.0336	0.033	78	Add.	0.0432	0.0426

TABLE 3.—CONCENTRATION OF UREA IN THE BLOOD OF NORMAL FASTING ADULTS—(*Continued*)

ARRANGED IN ORDER OF ASCENDING MAGNITUDE; DUPLICATE ESTIMATIONS

No.	Subject	Blood Concentration in 100 C.c.		No.	Subject	Blood Concentration in 100 C.c.	
79	Mo.	0.0435		93	Boy	0.0468	0.0462
80	Sha.	0.0435		94	Mo.	0.0485	
81	Add.	0.0438	0.0426	95	Sh.	0.0488	0.0456
82	Mon.	0.0438		96	Add.	0.0489	
83	Cal.	0.045	0.0444	97	Add.	0.0498	0.0498
84	Cal.	0.045		98	Mo.	0.051	0.051
85	Add.	0.045		99	Mon.	0.051	0.051
86	Add.	0.045	0.0428	100	Add.	0.0516	0.0516
87	Add.	0.045	0.0444	101	Add.	0.0522	0.0504
88	Mo	0.0462	0.045	102	Add.	0.0528	0.0516
89	Add.	0.0462	0.0462	103	Sha.	0.0547	
90	Add.	0.0462	0.045	104	Add.	0.0564	0.0552
91	Add.	0.0462	0.045	105	Wy.	0.0598	
92	Add.	0.0465		106	Add.	0.06	0.0598

carried out. The results are arranged in order of ascending magnitude in Table 3.

The average of this series is 0.038 per cent., while the average of the series on the third day of the diet was 0.0251 per cent. The increase was to be expected, since the diet contained only 75 gm. of protein—somewhat less than the average protein intake of healthy subjects. The range of variation—from 0.0225 to 0.0600 per cent.—is also greater, as was to be expected from the greater variability of the conditions.

The most striking and interesting point in the table is the demonstration of the fact that the variation in one person is as great as the variation in the whole group. Fifty out of these 106 estimations were carried out on one subject (Add.). His blood concentration varied from 0.0246 to 0.06 per cent., which is practically the group variation. This was not the result of wide fluctuations in protein intake on the days before the blood was taken, for this subject habitually took a very moderate and not unusually inconstant amount of protein food. Nor is it an isolated instance of a special tendency toward inconstancy in the level of blood urea, for the subject Mon., on whom only eleven estimations were made, also shows the wide variation of from 0.027 to 0.051 per cent. This is a strong argument against the view that the variation in urea concentration in one subject as compared with another is likely in any of our cases to be

conditioned by any such permanent individual peculiarities as might be produced by structural differences in their kidneys. For if the same subject shows as great a variability as is manifested by the whole group, the cause of the group variability must be sought, not in any fixed differences in the kidneys, which regulate the blood concentration, but in an inconstancy of the conditions under which these kidneys were placed. We have shown that even when all dietary inconstancies are removed, there is still a high degree of variability. The variability in this series is therefore not wholly nor even mainly explained by differences in food intake. It is due, we think, for the most part to inconstancy in the rate of formation of urea in the processes of protein catabolism. During the two years during which these fifty observations were made on the subject Add. he may have passed through phases of decrease and increase in protein catabolism which might account for the wide variation in blood urea concentration which was found.

If changes in the rate of protein catabolism under physiologic conditions are accompanied by considerable fluctuations in the level of blood urea concentration, one would expect to find a still wider variation arising from the exaggerated disturbances in protein metabolism which occur in certain pathologic states. A marked increase in the concentration of nonprotein nitrogen or of urea in the blood has been noted in some diseases which are not necessarily associated with any renal deficiency. These include certain cases of pneumonia[8] and carcinoma,[9] as well as intestinal obstruction,[10] protease poisoning[11] and hyperthyroidism.[12] These are eminently conditions in which an increased protein catabolism may be expected.

In clinical work, blood urea estimations are at present usually carried out in order to determine whether or not there is an insufficiency in kidney function. It is obvious that the wide range of variation which exists precludes such determinations from being taken as an accurate measure of kidney function. Still the very highest levels of blood urea concentration are apparently only attainable when kidney elimination is defective. Concentrations above 0.15 per cent. speak decisively for renal decompensation. But below that figure judgment will be required in every case. A concentration of 0.1 per cent. may be very strong evidence of renal deficiency in one case, while in another in which there is reason to expect an increased rate of protein catabolism or in which unusual dietary or other conditions are present it may not justify more than a suspicion.

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8. Herter: Johns Hopkins Hosp. Rep., 1900, **9**, 69.
9. Strauss, quoted by Monakow, P.: Deutsch. Arch. f. klin. Med., 1914, **115**, 47.
10. Tileston, W., and Comfort, C. W.: THE ARCHIVES INT. MED., 1914, **14**, 620.
11. Whipple: Jour. Am. Med. Assn., 1915, **65**, 476.
12. Schwartz, H., and McGill, C.: THE ARCHIVES INT. MED., 1916, **17**, 42.

CONCLUSIONS

1. Differences in diet are a cause of variation in the concentration of urea in the blood of normal persons.

A change from a mainly carbohydrate to a protein-fat diet was accompanied by an increase of from 58 to 250 per cent. in blood urea concentration.

2. On a constant diet a variation of from 0.0156 to 0.0438 gm. urea per 100 c.c. of blood was found in twenty-nine experiments on twenty-five normal persons.

3. Differences in the rate of protein catabolism are the principal cause of the variation which was found in the blood urea concentration of normal persons on a constant diet.

The subjects who had the greatest rate of protein catabolism had the highest blood urea concentrations, while in those subjects in whom protein catabolism was least the blood urea concentration was lowest.

4. The blood urea concentration of normal persons is not maintained at a constant level by a proportionate increase in the rate of excretion of urea from the blood by the kidneys, whenever there is an increase in the rate of entrance of urea from the tissues into the blood. Although under such circumstances an increase in the rate of urea excretion occurs, it is not sufficient to prevent some rise in the blood urea concentration. This rise takes place whether the increased rate of entry of urea from the tissues into the blood is produced by a greater formation of urea from protein taken as food or from the breaking down of tissue protein or from the absorption of preformed urea from the alimentary tract.

5. Under inconstant conditions variation in blood urea concentration may be caused by alterations in the activity of the kidneys.

The more constant the conditions, the more uniform is the action of the kidneys in responding to a rise in blood urea concentration by a definite though not directly proportional increase in the rate of urea excretion; and there is reason to believe that if all the conditions could be kept constant, no fluctuations in blood urea concentration would arise from any inconstancy in the function of the kidneys themselves. But under widely different conditions it can be seen that the kidneys, even of the same person, do not act in a uniform manner, so that the same rise in blood urea concentration may lead to a greater rate of excretion under certain conditions than it will under others.

6. Permanent individual peculiarities play no part as a cause of variation in the blood urea concentration of different normal persons.

In a group of twenty-two subjects a variation of from 0.0225 to 0.06 gm. urea per 100 c.c. of blood was found in a series of 106 estimations, carried out in the morning before breakfast. Practically the same degree of variation was shown by one of these subjects, on whom fifty estimations were made.

AUTOTRANSPLANTATION AND HOMO-TRANSPLANTATION

OF THE THYROID GLAND USING THE THYROID CAPSULE AS THE SEAT OF TRANSPLANTATION *

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Having under our care a large number of cretins in whom cessation of the thyroid feeding leads rapidly to a recurrence of myxedema with its train of symptoms, we were led to undertake the following experimental work on autotransplantation and homotransplantation of the thyroid gland in the hope of obtaining sufficient positive results to warrant clinical operative interference. The following experimental operations are presented as a preliminary report.

AUTOTRANSPLANTATION

We have found that the autotransplants of the thyroid gland into the thyroid capsule was successful in all our cases, corroborating the work of other investigators in autotransplantation.

We have studied three such cases of multiple autotransplantation.

HOMOTRANSPLANTATION

The homotransplantation of the thyroid gland in animals is of great importance, and yet it has been unsuccessful up to the present time. Success in this form of transplant work would be a great step in solving the question of homotransplantation in general. The tendency of nearly all the workers, especially Manley and Marine,[1] von Eiselberg,[2] Payr,[3] Hesselberg,[4] Salzer,[5] Sermann[6] and Enderlen,[7] has been to

* Submitted for publication Oct. 11, 1916.

1. Manley, O. T., and Marine, David: The Transplantation of Ductless Glands, with Reference to Ferments and Function, Jour. Am. Med. Assn., 1916, **67,** 260.
2. Von Eiselberg: Transplantation of Thyroid and Parathyroid Gland, Arch. f. klin. Chir., 1914, **106,** 1.
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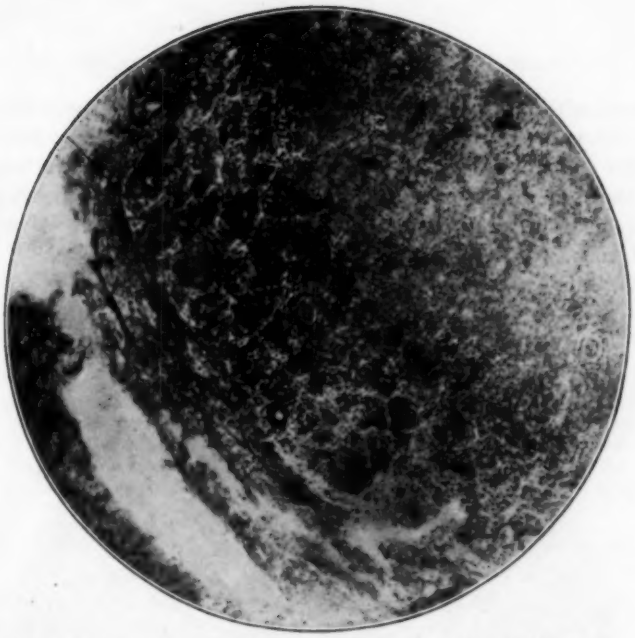

Fig. 1.—Autotransplant into loose areolar tissue in the neighborhood of the thyroid gland, twenty-five days after operation; magnification 15 diameters.

Fig. 2.—Same as Figure 1, magnification 60 diameters.

transplant the thyroid gland into some other portion of the body than into its normal location. Considering the highly specialized function of the organs of internal secretion, it seemed to us that the normal location was the best for definite functional reasons and therefore it seemed logical to us that we should attempt to transplant the organ in so far as possible at the seat of its normal blood supply.

We believe some of the important factors involved in a consideration of the normal position of this organ are, as follows:

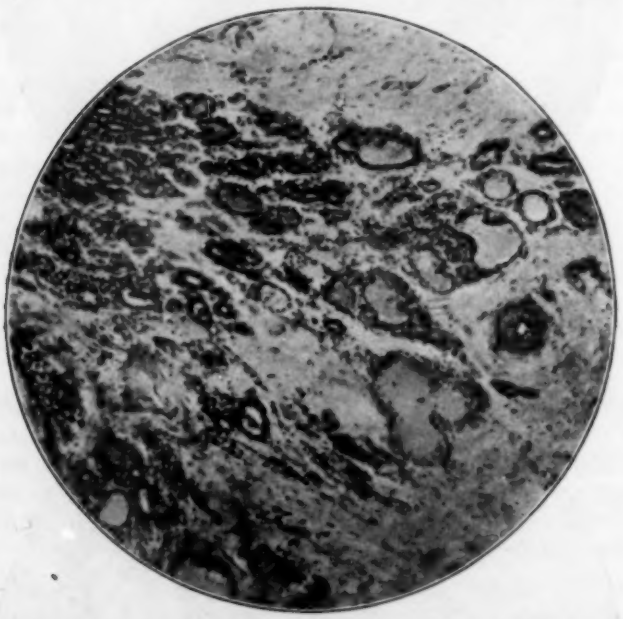

Fig. 3.—Autotransplant into capsule of thyroid gland, sixty days after operation; magnification 230 diameters.

1. The relation of the blood pressure and the size of the blood vessels nourishing this highly specialized organ must have an important bearing on the osmotic pressure of the blood and lymph as well as on the cellular structure of this particular organ, and consequently on its physiology.

2. It would appear that the chemistry of the tissue surrounding the thyroid transplant in intracapsular transplantation ought to be more adapted to its growth than would be the chemical reaction in foreign surroundings.

3. The secretion and iodin content of the remnants of the thyroid fragments within the capsule may be a factor in the retardation of the reaction between host and transplant in a manner similar to the results as shown by Marine, and Manley and Lenhart[8] in their iodin feeding of the host before the transplantation.

A considerable amount of experimental work has been done which, we believe, is conclusive proof that the quantity of secretion of the thyroid gland is regulated by stimuli reaching it through the circulation. Manley and Marine make the following statement in regard to their functional results in autotransplantation:

> Inasmuch as such transplanted thyroid tissue undergoes all the morphologic variations associated with growth and function that are observed in nontransplanted thyroid tissue, and inasmuch as transplanted thyroid tissue shows the same reaction with iodin and the same storage of iodin as nontransplanted thyroid, we believe that this is sufficient evidence that such transplants may grow, involute or function equally as well as nontransplanted thyroid gland.

The above quoted experiments lead us to the same conclusions as these authors, to the effect that specific nerves, whether secretory or regulatory, are not necessary for normal growth or functional activity of the thyroid gland.

TECHNIC

The next important point in this series of transplants was the preservation of the normal blood supply of the thyroid gland of the host. We attempted to do all our transplant work without cutting off any portion of the blood supply to the gland. This was accomplished by placing our transplants into the thyroid capsule without ligation of any of the vessels supplying the thyroid gland. We accomplished this by splitting the thyroid capsule and removing part, or in some cases practically all of the thyroid tissue. During this time the assistant secured both poles of the thyroid gland between the thumb and index fingers, in this way controlling the hemorrhage without severely traumatizing the intima of the large blood vessels and at the same time establishing a soft thrombus. At the end of eight to ten minutes we found that there was practically no bleeding, and we were ready to insert our transplant into its new bed formed by the thyroid capsule. We believe that the avoiding of injury by clamps and ligatures applied to the vessels supplying the thyroid of the host is of great importance to the transplants. Necropsy showed a complete restoration of the circulation through these vessels in every case examined.

In almost all of the operations we avoided getting the sutures near

8. Marine, David, and Lenhart, C. H.: On the Effects of the Administration or the Withholding of Iodin Containing Compounds in Normal, Colloid or Actively Hyperplastic Thyroids of Dogs, THE ARCHIVES INT. MED., 1909, **3**, 66; ibid., **4**, 253; ibid., **4**, 440.

the transplanted portion in our closure of the capsule, for it is well known that any foreign body will cause a local leukocytic reaction and connective tissue hyperplasia about such a foreign body; therefore, our sutures were placed either in the capsule or the surrounding connective tissue as far from the transplant as was possible.

We used dogs and guinea-pigs as experimental animals, and performed three autotransplants and thirty-one homotransplants, twenty-seven of these being done on dogs and four on guinea-pigs, applying the above technic in the hope of producing successful results.

Fig. 4.—Homotransplant in puppy 6 weeks old, necropsy 120 days after operation; magnification 60 diameters.

After doing three autotransplants and fourteen homotransplants, using the foregoing operative technic but without paying any attention to the age and familial relationship of the experimental animals or to the type of gland as seen in the individual animals (and it should be remembered that the thyroids of dogs are subject to great histologic variation), we found that the homotransplants had practically disappeared within twenty to thirty days, with the exception of two cases, in which small islands of thyroid tissue remained as isolated areas surrounded by connective tissue. These latter were not of such size as

to offer any considerable encouragement as to the feasibility in human subjects.

We can corroborate the work of Cora Hesselberg, who describes these homotransplants as a degeneration and necrosis in the central portion of the transplant, with an infiltration of leukocytes, lymphocytes and connective tissue cells, which takes place in a varying degree in various animals, depending on the reaction that exists between the host and the tissue used for transplantation.

Believing that age, familial and histologic variations might be important factors in accounting for the failures with the technic given

Fig. 5.—Homotransplant in puppy 7 weeks old, necropsy seventh day following operation; magnification 45 diameters.

above, we resorted to the use of young puppies of the same litter, aged from 2 to 6 weeks. We operated on eight puppies, using in each case those from the same litter. Seven of these puppies survived and were necropsied 7, 8, 19, 25, 60, 60 and 120 days, respectively, after the operation. In four of these we obtained living homotransplants, which can be described as follows:

There were small, isolated areas of thyroid alveoli, surrounded by new connective tissue or the capsule of the thyroid gland.

Many of the alveoli were seemingly normal in size. The cells were high and cuboidal in shape. Their nuclei stained well and the arrangement of the alveoli was normal. Many of the alveoli were filled with colloid material.

Dog 23 (Fig. 4), a female puppy, which was 6 weeks old when operated on, nursed its mother for several days following the operation and necropsy was performed 120 days after the operation. The transplant in this case was taken from a male puppy of the same litter. The surviving islands of thyroid tissue were embedded in areolar connective

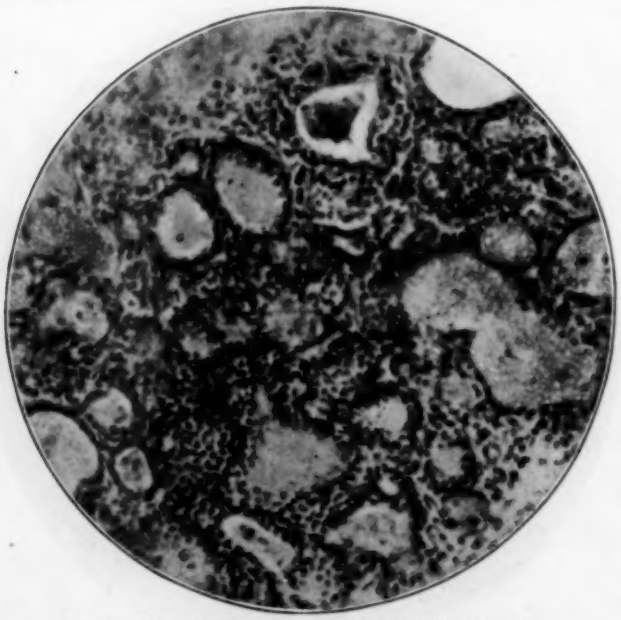

Fig. 6.—Same as Figure 5, magnification 230 diameters.

tissue, which was well organized with the remainder of the thyroid and connective tissue of the capsule proper.

Dog 24, a puppy, aged 7 weeks, was brought to necropsy on the seventh day following operation. The transplant had been taken from another puppy of the same litter (Figs. 5 and 6). There was a large area of transplant which showed quite marked degenerative changes and invasion of leukocytes and lymphocytes. At one side of this transplant there seemed to be a well-defined area of fairly normal-appearing thyroid tissue, which had the typical form of alveoli, filled with colloid material. The cells themselves were somewhat flattened, but the nuclei

stained very clearly and distinctly. This transplant showed changes similar to those seen in Dog 25, with the exception that there was not so much living thyroid in evidence.

Dog 25 (Figs. 7 and 8), a puppy, aged 7 weeks, of the same litter as Dog 24, from which the transplants were taken, was brought to necropsy on the eighth day. The transplanted thyroid showed a considerable hemorrhage, but not a great deal of infiltration of leukocytes and lymphocytes. There was a large part of this transplant which

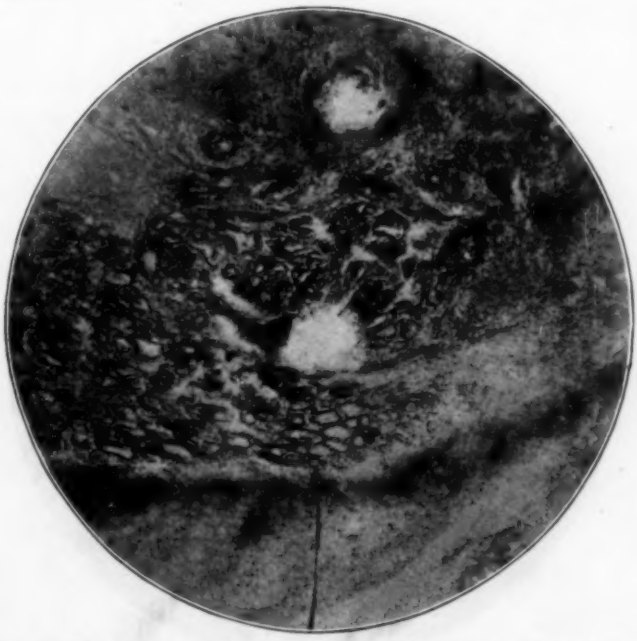

Fig. 7.—Homotransplant in puppy 7 weeks old, necropsy eight days after operation; magnification 50 diameters.

appeared almost normal. The alveoli of the living tissue were well arranged. Some of these alveoli were filled with colloid material. The nuclei stained well, but the cells were slightly flattened. Around the periphery of this transplant there was a large infiltration of leukocytes and lymphocytes. We believe that this thyroid transplant was living and well organized, but showed degenerative changes which were marked by the small alveoli and the flattening of the cells.

Dog 18, a puppy, aged 4 weeks (Fig. 9), was brought to necropsy sixty days after operation. A pocket had been made in the gland with-

out removing any of the capsule, and a piece of thyroid 1 by 2 by 4 mm. from a pup of the same litter had been planted into the capsule, which had been closed without suturing through the transplant. At necropsy the transplant showed some degenerative changes, some hemorrhagic areas, and some lymphoid and leukocytic infiltration, but there were many small, isolated areas of thyroid tissue, which, however, had lost their alveolar arrangement. There were a few alveoli present in this mass which showed a small amount of colloid. The thyroid cells in this mass stained perfectly and the nuclei were very distinct. This area lay just beneath the suture line.

Fig. 8.—Same as Figure 7, magnification 230 diameters.

In the other three no such positive result was demonstrable. In two of these dogs, Puppies 16 and 17, the transplant was destroyed in sectioning the tissues.

In the four puppies described which were operated on with tissues taken from puppies of the same litter there seemed to be less destructive reaction between the host and the tissue transplanted into the thyroid gland than in the older dogs from different litters, and therefore these transplants survived in a manner similar to that seen in autotransplantation, but with much smaller islands of living tissue. The

results shown in Figs. 5, 6, 7 and 8 may be open to question because of the short time between operation and necropsy, seven and eight days, respectively. However, we have based our conclusions on the well-preserved sections of tissues as shown in photomicrographs.

TRANSPLANTATION AFTER REPEATED PRELIMINARY TRANSFUSION

We cross-transfused several animals over a period of several weeks, giving from two to four injections at intervals of one week, using from 40 to 100 c.c. of freshly drawn citrated blood from each animal, which

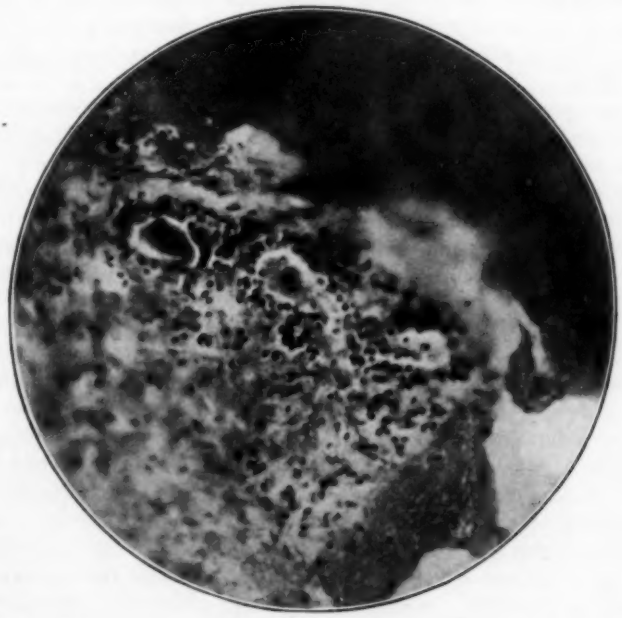

Fig. 9.—Homotransplant in puppy 4 weeks old, necropsy sixty days after operation; magnification 230 diameters.

was rapidly injected intravenously. Following this preparation, we cross-transplanted the thyroid glands, in each instance using the thyroid capsule as the seat of the transplant. Tests were made for the production of isohemolysins and iso-agglutinins in the two sets of dogs operated on, following two preparatory transfusions in one set and three in another set of dogs, but none were found.

We obtained the same results as Lexer and Morris, namely, this seemed to increase the reaction between the host and the transplanted tissue and thereby hastened the destruction of our transplants. It seems

to us, therefore, that to prepare the animal for transplantation would mean to prepare the blood and tissues in such a way that a leukopenia rather than a leukocytosis would be produced in the region of the transplant. To produce this seems to be the stumbling block in the efficiency of the homotransplant work.

CONCLUSIONS

We believe that the transplantation of autotransplants and homotransplants of highly specialized organs into the region normally occupied by these tissues is worthy of further trial.

We believe there should be a minimum disturbance of the blood supply in the region in which the transplant is placed, and that foreign bodies, such as suture material, should not come into contact with the transplant.

The varying degree to which a homotransplant takes depends on the amount of reaction between the host and the tissue transplanted, and so far we have found no means either in the blood or in the thyroid gland itself by which we could determine the factor which produces this different condition in the various animals.

A familial relationship and probably the early age of the animals on which operation was performed were important factors in our results.

We desire to thank Dr. J. J. Moore of the University of Illinois College of Medicine for the serologic work embodied in this article.

THE EFFECT OF SALICYLATES ON EXPERIMENTAL ARTHRITIS IN RABBITS*

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In a study of this question by Dr. D. J. Davis,[1] he arrives at the following conclusion:

> Sodium salicylate does not exert a favorable effect on infections in rabbits caused by various types of streptococci. It does not prevent localization of the organism in joints, nor does it prevent the appearance of endocarditis. It would seem to have, therefore, no prophylactic value, nor does it alter the course of the infection after it has once become established.

In these experiments sodium salicylate was used in a dose of 0.3 gm. administered subcutaneously once daily. In view of the great importance of the question, and the fact that the subcutaneous administration of a single daily dose by no means reproduces the usual therapeutic administration of this remedy in human beings, and as only large doses produce marked effect in human rheumatism, and the daily dose of 0.3 gm. does not represent the limit of possible dosage in the rabbit, it was thought desirable to repeat this work using a larger dose and employing various means of administration of the salicylate so as to maintain, as far as practicable, a fairly high and continuous salicylization of the system. This represents the modern ideal of administration of salicylates in the treatment of human rheumatism, based on the assumption that salicylate acts in this condition as a systemic antiseptic, not as disinfectant. It is generally agreed, as a result of clinical experience, that salicylate cannot exterminate the causative organism of rheumatic fever in the system of the patient. All that it is believed we can accomplish with salicylate is to inhibit the proliferation of the micro-organisms in the joints; and, to obtain this result, we generally need as large doses as the patient can tolerate, administered as continuously as possible for a considerable length of time. It is obvious that the experiments just cited, in which a single daily dose was administered, merely proved that the dose used was insufficient to exterminate or harm the streptococci. Such dose administered subcutaneously

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* From the departments of Therapeutics and Experimental Medicine, University of Illinois College of Medicine.

1. Davis, D. J.: The Effect of Sodium Salicylate on Various Types of Experimental Arthritis, THE ARCHIVES INT. MED., 1915, **15**, 555.

is rapidly absorbed, floods the system with a wave of salicylate, which, however, soon subsides owing to rapidity of elimination. That such treatment was ineffective proved by no means that the continuous maintenance of as high a level of salicylization of the system as possible might not have been beneficial to rabbits infected with the arthritis-producing streptococcus isolated from human patients suffering with rheumatic fever. In the work undertaken by us we attempted to induce the conditions just specified, to determine whether we could obtain any evidence of the assumed systemic antiseptic action of salicylates.

TABLE 1.—Sodium Salicylate 0.5 Gm. Twice Daily

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
Salicylate Controls	(Capsules)	(Solution)			
S 13	+ 45	— 20	....	.....	Survived
S 14	+ 5	+ 30	....	.....	Survived
S 15	+ 55	+ 7	....	.....	Survived
	Av. + 35	Av. + 6			
Sodium Salicylate and Hemolytic Streptococcus	(Solution)				
Rabbit 3	— 84 in 3 days		1	Gastric	Lived 3 days*
Rabbit 5	— 85 in 4 days		1	Gastric congestion	Lived 4 days
Rabbit 11	— 44 in 3 days		1	Gastric congestion	Lived 3 days
Hemolytic Streptococcus Controls					
Rabbit 7	+ 62	+ 95	5	1	Survived
Rabbit 10	— 34	— 98	8	2	Survived
Rabbit 12	— 44	—118	5	1	Survived

* The animals treated with sodium salicylate all died, while the untreated animals recovered. That the salicylate did not kill the animals is shown by the negative result of the salicylate controls.

SODIUM SALICYLATE

Young rabbits weighing about 1,000 gm. were used in our experiments. The effect of the drug it was proposed to use in treatment was first studied by administering it to normal animals. Table 1 shows that when sodium salicylate was given in doses of 0.5 gm. twice daily by the stomach, it produced no markedly unfavorable results in the rabbits, excepting to retard their growth somewhat. It will be noted that administration in capsule form, used in the first week, was less unfavorable to growth than administration in solution (in 10 c.c. of water) practiced by means of the stomach tube during the second week. Nevertheless the difference was not great. Inasmuch as it is difficult to be sure that the animal gets the whole of the medicament when it is given in capsule, as rabbits chew the capsule and are likely

to reject a portion of the drug in spite of all possible care taken to prevent this, administration in solution was used in the treatment of the infected rabbits. The rabbits to be treated were injected by way of the ear vein at about the same time with equal-sized doses of the same culture of hemolytic streptococcus isolated from the tonsil of a patient suffering from rheumatic polyarthritis. It will be seen that all the treated rabbits died in from three to four days, while all the untreated animals survived though they had a course of fever and developed arthritic lesions. That the treated rabbits showed no joint lesions and had but one day of fever is not to be put to the credit of

TABLE 2.—EFFECT OF SODIUM SALICYLATE 0.3 GM. ADMINISTERED HYPODERMICALLY ONCE DAILY *

Infected Controls			Infected Animals Treated with Sod. Salicylate†		
Experiment	Rabbit	Lived, Days	Experiment	Rabbit	Lived, Days
1	1	8	1	4	4
1	2	11	1	5	7
1	3	7	1	6	3
2	1	5	2	4	5
2	2	3	2	5	2
2	3	Survived	2	6	3
3	1	Survived	3	4	9
3	2	Survived	3	5	Survived
3	3	Survived	3	6	Survived
4	1	4	4	4	5
4	2	5	4	5	8
4	3	Survived	4	6	9
		5 survived 7 died			2 survived 10 died

* Experiments of D. J. Davis.
† The influence of the salicylate was certainly not favorable.

salicylate, for they died before joint involvement and fever had time to develop. We must conclude that the administration of sodium salicylate was fatal to the infected rabbits, when used in a dose that did not prove very detrimental to the normal controls.

It may be of interest here to tabulate the final results of the experiments reported by Davis.[1] It will be seen by consulting Table 2, that while 60 per cent. of the infected, untreated animals died in an average of six days, 83 per cent. of the treated rabbits died in an average of 5.5 days. These figures evidently point in the same direction as those of Table 1, namely, that sodium salicylate harms rather than helps rabbits infected with streptococcus. When the dose of the salicylate

TABLE 3.—Sodium Salicylate 0.3 Gm. and Sodium Bicarbonate 0.3 Gm. in Water 15 C.c. Three Times a Day

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
Salicylate Bicarbonate Controls	(Capsules)	(Solution)			
S 2	— 15	+ 45	....	.....	Survived
S 4	+ 5	—200	....	.....	Survived
S 10	+ 40	+ 58	....	.....	Survived
S 11	+ 70	+ 84	....	.....	Survived
S 0	—126	— 79	....	.....	Survived
	Av. — 7	Av. — 20			All survived
Salicylate and Bicarbonate* and Hemolytic Streptococcus	(Solution)				
Exp. 1, Rabbit 22	— 70	.....	5	2 Pneu.	Lived 9 days
Rabbit 24	+ 25	— 6	11	1	Survived
Rabbit 28	—164	.....	6	3 Pneu.	Lived 9 days
Exp. 2, Rabbit 41	— 60	.....	6	4	Lived 7 days
Rabbit 42	— 98	—133	10	4	Survived
Rabbit 43	— 34	.....	1	6	Lived 6 days
Exp. 3, Rabbit 47	— 55	—155	5	4	Survived
Rabbit 48	—122	.....	0	0	Lived 4 days
Rabbit 49	+ 28	— 86	6	Pul. C. 4	Survived
	Av. — 62	Av. — 95	Av. 5.6	Av. 3.5	5 died 4 survived
Hemolytic Streptococcus Controls					
Exp. 1, Rabbit 16	+ 30	+240	4	2	Survived
Rabbit 17	+ 0	+110	8	0	Survived
Rabbit 23	+ 83	+261	3	1	Survived
Exp. 2, Rabbit 31	— 33	— 28	10	4	Survived
Rabbit 32	— 98	—135	7	6	Survived
Rabbit 45	+ 45	—137	10	4	Survived
Exp. 3, Rabbit 60	— 17	— 23	11	4	Survived
Rabbit 61	— 31	— 47	12	2	Survived
Rabbit 62	—104	—204	6	5	Survived
	Av. — 14	Av. — 4	Av. 7.9	Av. 3.5	All survived

* Of the animals treated with sodium salicylate and sodium bicarbonate more than half the number died, while all the untreated, infected animals, as well as the medicated controls, survived.

was smaller or when the infections were more virulent, the unfavorable effect of the drug was less apparent.

SODIUM SALICYLATE AND SODIUM BICARBONATE

Inasmuch as sodium salicylate is generally administered with alkali, it seemed desirable to make a study of the effect of the combination on normal and infected rabbits. All the five normal controls (Table 3), which were given 0.3 gm. each of sodium salicylate and sodium bicarbonate three times daily, survived. The only harm the treatment did was that some of the animals lost in weight instead of gaining. All the nine untreated, infected animals survived, though all of them had a course of fever (average number of fever days about eight), almost all of them developed lesions (average number 3.3), and all of them lost in weight, excepting the animals used in Experiment 1, all of which gained in weight. This, as well as the other figures in connection with Experiment 1, shows that the virulence of the organisms used was comparatively low. In spite of this, the treated animals in Experiment 1, which were infected with the same kind of organism, had a much more unfavorable course, as will be seen by a glance at Table 3. Summarizing the three parallel experiments, we find that 55 per cent. of the treated animals died. Even though we discount the deaths with pulmonary lesions as possibly accidental, the fact still remains that the average number of lesions is greater, even though one of the treated animals (Rabbit 48) died before there was time for the development of lesions. The smaller average number of fever days (5.6) must not be interpreted as indicating an antipyretic action of the salicylates, for the four treated animals that survived had an average of eight fever days, which is the same as that of the untreated animals. Dying animals often have a low temperature for several days before death. The conclusion is inevitable that the treatment was harmful to the infected animals.

To determine the effect of sodium bicarbonate on normal and infected rabbits, to see what share it might have had in the unfavorable results produced by the combination with salicylate, an experiment was conducted, the results of which are shown in Table 4. Sodium bicarbonate is at least not harmful to the sick animals, though it certainly did not produce a noticeable improvement.

ACETYLSALICYLIC ACID

Owing to the popularity of acetylsalicylic acid it was thought desirable to try it in an experiment; 0.33 gm. of it, which is approximately equal in salicylic acid content to 0.3 gm. of sodium salicylate, was given three times daily. The results were surprisingly bad, even in

TABLE 4.—Effect of Sodium Bicarbonate 0.6 Gm. Three Times Daily

	Gain or Loss in Weight in Two Weeks, Gm.	Fever Days	Lesions
Sodium Bicarbonate Controls			
Rabbit 62	+125		
Rabbit 3	— 60		
Rabbit 59	—136		
	Av. — 20		
Sodium Bicarbonate* and Hemolytic Streptococcus			
Rabbit 121	—125	4	4
Rabbit 130	—205	5	6
Rabbit 131	—125	3	6
Rabbit 132	—115	6	7
	Av. —162	Av. 4.5	Av. 5.75
Hemolytic Streptococcus Controls			
Rabbit 134	—140	7	0
Rabbit 135	—297	5	14
Rabbit 136	—130	6	2
Rabbit 137	—105	3	2
	Av. —183	Av. 5.25	Av. 4.5

* Sodium bicarbonate did not produce a noticeable improvement in the infected rabbits.

TABLE 5.—Effect of Acetylsalicylic Acid 0.33 Gm. Three Times Daily*

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
Acetylsalicylic Acid Controls					
Rabbit 30	— 15	—160	....	Kidney pale	Lived 15 days
Rabbit 33	+ 35	—124	....	Kidney pale	Lived 16 days
Rabbit 34	—125	(—125)	....	Pneumonia	Lived 11 days
	Av. — 25	Av. —136			Aver. 15 days
Acetylsalicylic Acid and Hemolytic Strepto.					
Rabbit 146	—125	.....	4	15	Lived 8 days
Rabbit 147	—247	.....	5	17	Lived 8 days
Rabbit 148	—100	.....	4	15	Lived 6 days
Rabbit 149	—115	.....	3	17	Lived 5 days
	Av. —162		Av. 4	Av. 16	Aver. 7 days
Hemolytic Streptococcus Controls					
Rabbit 128	—130	(—130)	3	11	Lived 7 days
Rabbit 129	—220	—255	6	12	Lived 21 days
Rabbit 140	—125	—130	1	10	Lived 12 days
Rabbit 141	— 45	—168	5	3	Lived 21 days
	Av. —130	Av. —208	Av. 4	Av. 9	Aver. 15 days

* Acetylsalicylic acid proved quite toxic to the control animals, and definitely harmed the infected rabbits.

the control animals. Table 5, which gives the results of this experiment, shows that all the control animals died. If we disregard the one that died from pneumonia, we have two animals that seemed to show kidney involvement as a result of the administration of this substance. While all the infected animals died, for we had a virulent strain to deal with in this series, the treated animals died in half the time and showed almost twice as many lesions. Acetylsalicylic acid, in equivalent dosage, appears to be much more toxic to normal rabbits than is sodium salicylate and it is harmful to infected rabbits as well.

TABLE 6.—EFFECT OF SALOPHEN 1.5 GM. DAILY IN FOOD*

	Gain or Loss in Weight, Gm.		Fever, Days	Lesions	Final Result
	One Week	Two Weeks			
Salophen Controls					
Rabbit 1	— 41	+ 10	....	.....	Survived
Rabbit 2	— 47	+ 10	....	.....	Survived
Rabbit 4	— 0	+ 80	....	.....	Survived
Rabbit 10	— 5	+ 35	....	.....	Survived
	Av. — 23	Av. + 21			
Salophen and Hemolytic Streptococcus					
Rabbit 12	— 45	+ 30	1	4	Survived
Rabbit 64	—160	.....	4	8	Lived 9 days
Rabbit 101	—206	.....	4	12	Lived 6 days
	Av. —137		Av. 3	Av. 8	
Hemolytic Streptococcus Controls					
Rabbit 90	—130	(—130)	1	11	Lived 4 days
Rabbit 115	—172	—235	8	5	Survived
Rabbit 116	+ 3	+ 38	5	4	Survived
	Av. —101	Av. —109	Av. 5	Av. 7	

* Salophen, while it scarcely injured the control rabbits, harmed rather than helped the infected animals.

SALOPHEN

In all the experiments thus far reported the medicament was administered in capsule or by means of the stomach tube. In either case struggling of the animals was inevitable. To eliminate this factor, which might have an unfavorable influence on the sick animals, salophen in doses of 1.5 gm. daily, the equivalent to 0.9 gm. of sodium salicylate, was given in the food. Rabbits readily eat chopped carrots mixed with salophen, as this substance is practically tasteless. The only disadvantage of this method of administration consists in the fact that sick rabbits do not eat, and therefore do not get the medicine when it is mixed with the food. However, inasmuch as several days pass between the infection and the development of severe symptoms, the

rabbits certainly took a sufficient amount of medicine for it to have some influence on the course of the disease. That there was either no influence or else an unfavorable one is shown in Table 6.

COMMENT ON RESULTS

The results of the experiments reported do not permit one to escape the conclusion that in the experimentally induced arthritis of rabbits salicylates are not only worthless, but even harmful. To bring these results in harmony with the clinically established usefulness of salicylates in rheumatic fever in human beings several possibilities must be considered.

First, the *Streptococcus hemolyticus* used in these experiments might not have been the strain of streptococcus that is particularly susceptible to salicylate. It is well known that not all cases of infectious polyarthritis respond to salicylate in a specific manner. Some of these cases are quite as resistant to it as is gonococcus polyarthritis. It may therefore be desirable to repeat the experiment with a variety of different strains of streptococci. This is what Davis[1] did, with results that were distinctly unfavorable to the salicylate.

Second, it might be that there is a chemical difference between the system of the rabbit and that of man in its relation to salicylate, for example, a difference in the hydrogen ion concentration of the tissue fluids. If the action of salicylate in rheumatism depended, as has been suggested, on the liberation of salicylic acid in the inflamed tissues by reason of a high carbon dioxid tension in them, it might be that, owing to a greater alkalinity of the rabbit's tissue fluids, no salicylic acid is liberated in case of these animals. It would therefore be desirable to compare the reaction of the tissue fluids of rabbits with that of man. It would also be interesting to repeat these experiments on some other species of animals, such as young dogs or monkeys.

Third, the action of salicylate in human beings might be merely symptomatic, but not really curative. That there are clinicians who have this opinion is evidenced by such articles as Menzer's,[2] who, on the basis of personal observation of 140 patients, arrived at the conclusion that although salicylates may alleviate the symptoms to a marked degree, patients so treated are much more subject to recurrences, and in particular to deforming arthropathies, than were those handled without the use of salicylate. Miller,[3] on the basis of a rather extensive statistical study, concludes that "it would not appear justi-

2. Menzer, A.: Dienstunbrauchbarkeit und Rückfalle bei Behandlung des Akuten Gelenkrheumatismus mit und ohne Antipyrese, Ztschr. f. Hyg. u. Infectionskrankh., 1911, **68,** 296.

3. Miller, Joseph L.: The Specific Action of Salicylates in Acute Articular Rheumatism, Jour. Am. Med. Assn., 1914, **63,** 1107.

fiable to refer to the salicylates as a specific in acute articular rheumatism, but rather as a drug which modifies the course of the disease, without actually shortening the period of infection." "The treated patients much more frequently relapse than the untreated." Badt[4] reports 2.7 per cent. of deaths among 148 cases of rheumatism treated with salicylate as compared with 0.6 per cent. of deaths in 176 cases treated without salicylate. It may therefore be that even in the human patient, as in the rabbit, salicylate harms rather than helps.

CONCLUSIONS

1. Sodium salicylate, when used in a dose comparatively harmless to animal controls, is decidedly detrimental and liable to be fatal to animals infected with the hemolytic streptococcus.

2. The addition of sodium bicarbonate does not lessen much the harmfulness of the salicylate to infected rabbits.

3. Sodium bicarbonate given alone is not injurious to infected rabbits, neither does it produce a noticeable improvement.

4. Acetylsalicylic acid, in equivalent dosage, appears to be much more toxic to normal rabbits than is sodium salicylate, and is harmful to infected rabbits as well.

5. Salophen has either no influence or an unfavorable one on the course of the infection.

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4. Badt: Quoted by Menzer, Footnote 2.

ROENTGENOGRAPHY OF THE LUNGS

ROENTGENOGRAPHIC STUDIES IN LIVING ANIMALS AFTER INTRATRACHEAL INJECTION OF IODOFORM EMULSION *

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During the past thirty-two years, Rosenberg's[1] method of treating laryngeal and pulmonary tuberculosis by means of intratracheal injections of medicinal agents has been used to a rather limited extent only. In 1886, Sehrwald,[2] injecting into the trachea by "percutaneous punctures," showed that 10 to 20 c.c. of a mixture of olive oil and menthol could be thus injected without discomfort to the patient. Campbell[3] reported on the successful use of this method of treatment in 1895, describing cases in which he had injected as much as 100 c.c. of the oil-menthol mixture in twenty-four hours. The amount used by him at a single injection was usually 10 to 15 c.c. The results of this mode of treatment have not been sufficiently brilliant to obscure its possible dangers, and the method has not been employed extensively. The recent work of Auer and Gates[4] on the absorption of epinephrin (adrenalin) after intratracheal injection may give this method some of the clinical vogue which Sehrwald regarded as its legitimate due.

The studies to be reported in this paper were undertaken to acquire a clearer conception of the anatomy and physiology of the lungs in life. On the basis of the previous therapeutic use of relatively large intratracheal injections, a method was devised whereby the air passages could be filled or coated with material opaque to the Roentgen rays. As is well known, the interpretation of many shadows in Roentgen-ray plates of the lungs is limited only by the number of anatomic possibilities. It is often a matter of importance to determine whether lines in the plates represent bronchi, vessels, or fibrous tissue. Frequently, however, it is impossible to make this distinction. It is obvious that

* Submitted for publication Sept. 28, 1916.

* From the Medical Clinic and Roentgen Department of the James Buchanan Brady Urological Institute, Johns Hopkins Hospital.

* Read in part before the Society of Clinical Surgery, Baltimore, Oct. 20 and 21, 1916.

1. Rosenberg, A.: Berl. klin. Wchnschr., 1885, **22,** 449; ibid., 1887, **24,** 466.
2. Sehrwald: Deutsch. Arch. f. klin. Med., 1886, **39,** 162.
3. Campbell, C.: Med.-Chir. Trans. (London), 1895, **78,** 39.
4. Auer, J., and Gates, F. L.: Jour. Exper. Med., 1916, **23,** 757.

roentgenograms of lungs before and after the intratracheal injection of material impenetrable by the Roentgen ray would give information as to the anatomic condition of the lungs, and thereby supply helpful diagnostic data.

MATERIAL AND TECHNIC OF INJECTION

As iodoform has been used in the local treatment of pulmonary tuberculosis, in bronchiectasis, and in putrid bronchitis, and since it was known to cast a shadow, it was the first choice. Preliminary experimentation showed that a suspension of iodoform in olive oil in thin layers definitely impeded the Roentgen rays. As a result of investigation it was found that the most satisfactory material was a 10 per cent. suspension of iodoform in olive oil. Successful roentgenograms

Fig. 1 (Plate 1-A).—Shows the entire bronchial tree completely filled with iodoform emulsion. The injection has been disseminated out into the alveoli, giving the lungs a "fuzzy" appearance.

were obtained with ordinary iodoform in olive oil, but a better suspension was prepared by grinding up 10 gm. of finely-divided, silk-screened, chemically-pure iodoform in 100 c.c. of olive oil.[5] No difference was observable between various samples of olive oil, but in order to obviate any irritant action from this substance, it would be best to use olive oil freed from oleic acid. Iodoform emulsion of this consistency adheres to the bronchial walls and does not too completely fill the alveoli. This property is necessary in securing sharp photographs of the injected lungs.

5. Prepared by H. A. B. Dunning of the pharmaceutical firm of Hynson, Westcott & Dunning, Baltimore.

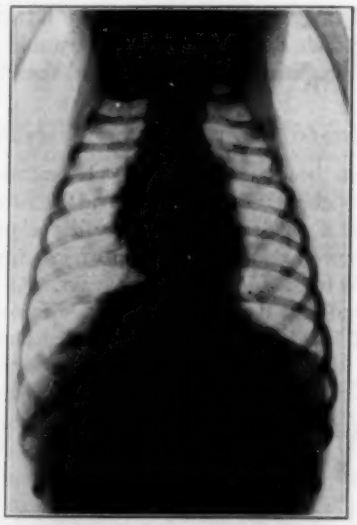

Fig. 2 (Plate 2-A).—Control; lungs before injection. Note peribronchial shadows in hilums of both lungs.

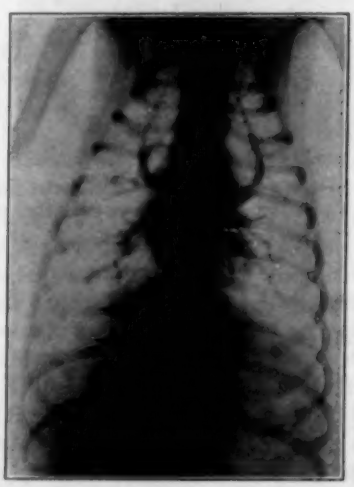

Fig. 3 (Plate 2-A).—After 10 c.c. were injected. The bronchi and bronchioles are clearly seen. This was the second injection after one month, and at the time of the injection the animal suffered from distemper, from which he died a few days later.

Fig. 4 (Plate 3-A, Control).—Indefinite shadows along the hilums of both lungs faintly seen.

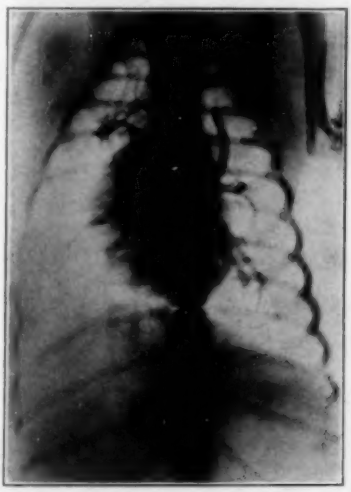

Fig. 5 (Plate 3-A).—After 7 c.c. had been injected. Note only the bronchi and bronchioles are filled. The animal survived.

Experiments were made with the solution of thorium recently described by Burns[6] as a valuable agent in pyelography. The shadows cast by this fluid in the air passages were too faint and diffuse for practical value. The fluid also seemed more toxic than the iodoform emulsion.

6. Burns, J. E.: Bull. Johns Hopkins Hosp., 1916, **27**, 157.

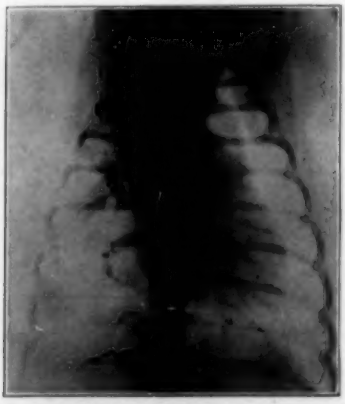

Fig. 6 (Plate 4-A).—Shows the right upper bronchi and alveoli well filled with iodoform emulsion. Dog choked to death.

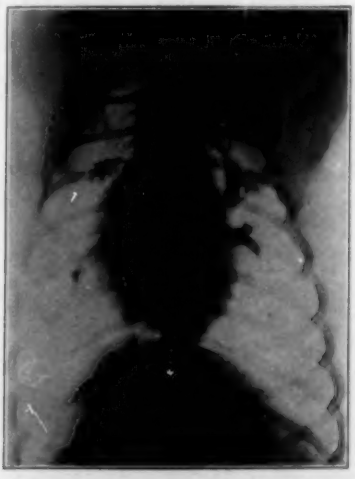

Fig. 7 (Plate 5-A).—The upper and middle bronchi well filled after 20 c.c. of the emulsion had been injected. No iodoform has passed into the alveoli.

The injections were made in the following manner:

The animal was deeply anesthetized with ether. With the animal (dog) on its back, its head retracted over the edge of the table and tongue drawn forward, a soft rubber catheter was passed through the glottis. This catheterization of the larynx is most easily made while the glottis is in full view. To secure this view a headlight was used. In order to maintain the tube in place a wooden block with a central hole for the reception of the catheter was placed between the animal's jaws. While the catheter was held in position the animal was turned on its abdomen and placed with its anterior thoracic wall

Fig. 8 (Plate 6-A).—Shows the entire bronchial tree outlined and the iodoform sticking to the wall of the upper right bronchioles.

Fig. 9 (Plate 7-A).—Shows animal weighing 18 pounds after 15 c.c. of iodoform had been injected, outlining the bronchial tree and alveoli. This was the second injection; the animal survived.

resting on a Roentgen-ray plate. At the proper moment the iodoform oil was injected forcefully through the catheter by a large syringe inserted into the outer end of the tubing. At the end of the injection an instantaneous exposure was made with a soft vacuum Roentgen-ray tube placed 20 to 30 inches above the plate.

DISTRIBUTION OF THE EMULSION IN THE LUNGS

When the lungs were dissected after an intratracheal injection of iodoform oil, this material, apparently in the same degree of suspension, was found in the bronchi, bronchioles and smallest air passages. Sections of the lungs fixed in 40 per cent. formaldehyd solution and stained with Scharlach R, showed large masses of fat filling the alveoli. The position of the catheter in the trachea determined somewhat the distribution of the fluid. As the plates show, however, the *bronchioles of the upper lobes and the alveoli of the apices were filled when the injection was made into the lower third of the trachea.*

Fig. 10 (Plate 10-A).—Shows lungs and bronchi completely filled after 25 c.c. iodoform emulsion was injected. Dog died in three minutes.

Stereoscopic roentgenograms were made on a number of these dogs, but owing to the retching following injection, it was quite difficult to keep the animal in the same position for the two exposures, and this method was soon abandoned as impracticable.

RESULTS OF INJECTIONS

It has been possible with this method to outline the entire bronchial tree, also to demonstrate that the alveoli can be injected approximately as easily as the bronchioles. The most satisfactory roentgenograms were obtained when 15 to 25 c.c. of the emulsion were injected.

Another interesting observation was the influence of position of the intratracheal tube in determining the distribution of emulsion to alveoli and bronchioles, the best results being obtained when the end of the

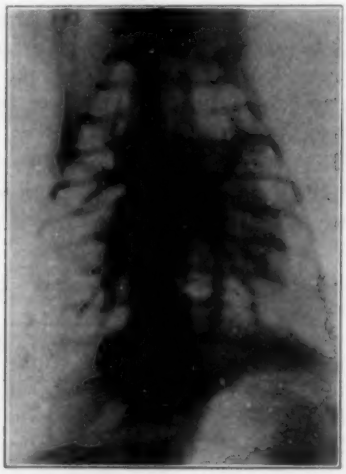

Fig. 11 (Plate 17-A).—Shows the bronchial tree completely filled after 40 c.c. injection. Dog died in three minutes.

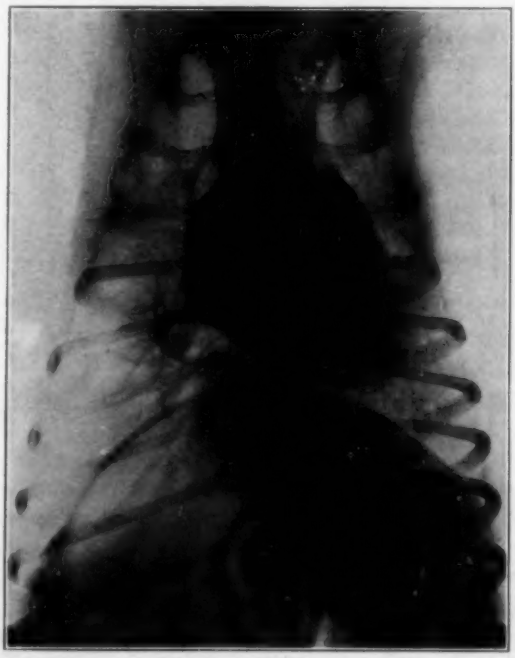

Fig. 12 (Plate 23-A).—Shows right upper and lower bronchi filled with 20 c.c. iodoform emulsion.

tube was just above the episternal notch. The bronchi leading to the apices and upper lobes were injected with as great frequency as the bronchi of the lower lobes, notwithstanding the difference in the anatomic relationship between the bronchioles of the upper and lower lobes to the course of the injection.

The accompanying table reveals the anatomic distribution of the injected fluid and shows the frequency with which each division of lung was injected.

TABLE SHOWING DISTRIBUTION—

Animals and X-Ray Plates	Position of Tracheal Tube	In Bronchi					
		Right Upper	Right Middle	Right Lower	Left Upper	Left Middle	Left Lower
1 - A	Medium	+	+	+	+	+	+
2 - A	Low	+	+	+	+	+	+
3 - A	High	+	+	+	+	+	+
4 - A	High	+	+	+	—	—	—
5 - A	Medium	+	+	—	+	+	—
6 - A	Low	+	+	+	+	+	+
7 - A	High	+	+	+	+	+	+
8 - A	Low	+	—	+	+	—	+
9 - A	High	—	—	+	—	—	+
10 - A	Medium	+	+	+	+	+	+
11 - A	Low	—	—	+	+	+	—
12 - A	High	—	—	+	+	+	+
13 - A	High	+	+	+	+	+	+
14 - A	High	+	+	+	+	—	+
15 - A	High	+	+	+	+	+	+
16 - A	High	—	—	+	—	—	—
17 - A	Low	+	—	+	+	+	+
23 - A	Low	+	—	+	—	—	—
23 - B	Low	+	—	+	—	+	+
23 - C	Low	+	—	+	+	+	+

EFFECTS OF THE INJECTION

Twenty-two living dogs were injected intratracheally with 10 per cent. iodoform oil. Of these, three were killed in determining the toxicity or "drowning" dose of the emulsion, three were killed ten days after an injection during an epidemic of distemper in the kennels, one was killed immediately after an injection for a dissection of the lungs, two died after injections of thorium, and three died of pneumonia probably directly attributable to the iodoform injection. Ten animals

survived more than a month after the injection, and some of these survived repeated injections. The mortality among the animals therefore appears to be approximately 25 per cent., or three out of thirteen.

The type of lesion in the lungs of the dogs whose death was considered to be due ultimately to the injected emulsion, was that of a proliferative bronchopneumonia. In all cases the exudate was found to be undergoing organization, while the alveolar and bronchial epithelium was considerably hypertrophied and in several instances

—OF INJECTED EMULSION

In Alveoli						Amount of Iodoform Oil Injected, C.c.	Weight of Dog, Kg.	Results
Right Upper	Right Middle	Right Lower	Left Upper	Left Middle	Left Lower			
+	+	+	+	+	+	25	2.7	Died in 3 minutes
—	—	—	—	—	—	10	..	Distemper when injected, died in 2 days
—	—	—	—	—	—	7	..	Survived
+	+	—	—	—	—	15	3.6	Choked to death
—	—	—	—	—	—	20	6.8	Distemper
+	+	+	—	—	—	15	7.3	Distemper
+	+	+	+	+	+	15	8.2	Survived 2 injections
—	—	—	—	—	—	8	4.5	Survived
—	—	+	—	—	—	5	4.1	Killed for pathologic study
+	+	+	+	+	+	25	..	Died in 3 minutes
—	—	+	—	—	—	15	Thorium 5.5	Pneumonia
—	—	+	+	+	+	20	Thorium 10	Died after 12 hours
+	+	+	+	+	+	15	8.2	Survived 2 injections
—	—	—	—	—	—	15	..	Survived
+	+	+	+	+	—	20	4.5	Survived
+	+	+	—	—	—	25	..	Died in 2 days—bronchopneumonia
—	—	—	—	—	—	40	..	Drowned
—	—	—	—	—	—	20	..	Dead when injected. See Plate 23A
+	—	+	+	—	+	40	..	See Plate 23B
+	—	+	+	+	+	60	..	See Plate 23C

heaped up in two or more layers of large epithelial cells. No giant cells were seen in the sections and there was no process to suggest that the iodoform had provoked a foreign-body type of reaction.

SUMMARY AND CONCLUSIONS

A method is described by which shadows cast by the air passages of the lungs may be unequivocally defined in roentgenograms. This method consists of injecting into the trachea a 10 per cent. suspension

Fig. 13 (Plate 23-B).—Shows same animal after 40 c.c. iodoform emulsion had been injected. The stippled shadows show that the injected material has filled up the alveoli.

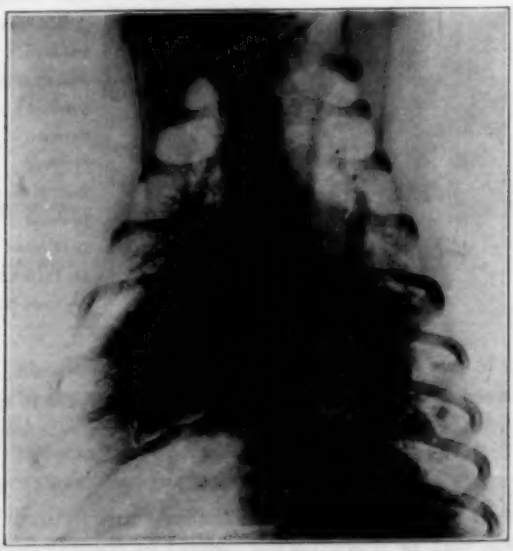

Fig. 14 (Plate 23-C).—Same animal after 60 c.c. had been injected. The entire bronchial tree with the alveoli are clearly shown filled with iodoform emulsion.

of iodoform in olive oil, which renders the air passages opaque to the Roentgen ray. The limit of a safe dose of this emulsion for dogs is found to be approximately 4 c.c. per kilogram of body weight. The plates are made by an instantaneous exposure by a soft vacuum tube placed above the animal (20 to 30 inches above the plate). As similar suspensions of iodoform in oil have been used in the local therapy of human pulmonary diseases, it is hoped that perfection of the method may ultimately permit its application to man. This preliminary study is the basis of the belief of the authors that by such a method it may become possible to define with precision many radiographic shadows which are at present largely data for speculation.

A CASE OF INFUNDIBULAR TUMOR IN A CHILD

CAUSING DIABETES INSIPIDUS WITH TOLERANCE OF ALCOHOL *

L. NEWMARK, M.D.
SAN FRANCISCO

The clinical notes in this case are deficient in a number of particulars which those interested would wish to know, and the anatomic examination could proceed but little beyond the parts primarily involved. But defective as the following description is, it is submitted as exhibiting a good example of a rather uncommon disorder. The tolerance of alcohol in diabetes insipidus is mentioned in textbooks, but it does not appear that their statements are based on any considerable body of observations. In the reports published in recent years of cases of the disease and of experimental polyuria in animals, this subject seems not to have been considered at all.

REPORT OF CASE

History.—According to information kindly supplied by Dr. Florence Scott of Belvedere, Calif., the patient, Pete B., a native of Italy, fell from a wagon when he was 4 years old and fractured his skull. He recovered completely from the effects of this accident, and it is considered certain that he did not have the unquenchable thirst before he was about 9 years old. Since the age of 6 he was addicted to smoking cigarets. To Dr. Florence Scott the child's polydipsia was revealed when she saw him at a comparatively late hour one night walking about the house with a large pitcher of water which he had left his bed to get. Inquiry elicited that the boy had been drinking very copiously and urinating large quantities every night for about a year previously. As he was at that time 10 years of age, the polyuria and polydipsia must have attracted the attention of his parents when he was about 9. His father subsequently told me that the boy drank as much as 2 gallons of water during the night, and that a bucket of a capacity of 4 gallons was placed in his bed room as a urinal and was regularly found half filled in the morning. The urine, the father added, looked like the water the child drank. Dr. Scott examined it many times and found the specific gravity generally 1.002 and never more than 1.006; sugar and albumin were always absent.

The impression obtained was that the thirst and the urinating were rather more troublesome at night than during the day; for, while there was abundant evidence that the boy drank copiously throughout the day (the customers, for instance, to whom he delivered milk, observing how frequently he drank at the faucet on his visits), yet his teacher related that he never had to be excused during lessons to drink or to urinate.

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* Submitted for publication Sept. 22, 1916.

* From the San Francisco Polyclinic. Thanks are due to Professor Ophüls of Stanford University, and to Professor Rusk of the University of California, for much help in pathologic matters, and to Dr. H. D'Arcy Power of the Polyclinic, for the photographs of sections.

There was no limit to the amount of water procurable to slake his thirst; but he craved stronger drink. On account of his early addiction to tobacco and a propensity to "swear dreadfully," he had for some time been viewed askance; and the measure of his iniquity seemed indeed full when the father denounced his son to Dr. Scott as a bad boy, incorrigibly given to drink. Severe beatings did not deter the boy from various tricks to obtain wine. He would drink the home-made wine from the barrel by sucking it up through a tube. To obtain beer from the grocer (who refused to sell it to him directly) he would hand his money to some vagrant and purchase through that agent. It was a matter of common knowledge in the neighborhood that P. would not recoil even from whisky. This was denied to me by the father after the child's death, but I am informed that in a recital of his complaints about the boy some years before, the drinking of whisky had been included. I have the father's word for it, however, that the boy would drink three or four glasses of beer in rapid succession, or toss off several glasses of wine. His indulgence in wine exceeded by far the measure of what some may think is permitted the young among people of Latin race. The father said he had never seen a child drink alcohol like Pete. But he had never seen the boy drunk, nor could any one else be found who had seen him affected by drink. After a bout, for instance, shared by "another little boy" who, we may safely presume, got but a small portion of the four quarts and a pint of beer consumed, he reported at school and performed his tasks well, as he usually did. His teacher rated his intellect as developed in proportion to his years, and approved his work to the last.

The headaches from which he suffered at intervals of several weeks for at least three years, were looked on as "sick headaches," and were not ascribed to indulgence in alcohol, or to grave organic disease. Generally they did not last beyond a day, and his attendance at school was not interrupted for a longer period than that, until about two weeks before his death. They were not always severe enough to make him take to his bed. He did not vomit in the attacks. The headaches over, he would resume his smoking and drinking whenever opportunity presented.

Examination.—In January, 1914, on a visit to the village where he lived, the subject of the foregoing history was brought to my attention as a "case of diabetes insipidus." The patient proved to be a weazen, alert and agile little boy, 14 years of age, but who looked not more than 10. He had not grown much, if at all, for a few years previously, and had always been considered small for his years. (His father is only 5 feet 5 inches in height.) On this occasion I examined the boy as well as the circumstances permitted. In view of the diabetes insipidus and its reference to lesions in or near the hypophysis, I tested especially the sense of smell, examined the backgrounds of the eyes, and sought to determine the visual fields. The result of the examination was negative.

Second Examination and Course.—Very different was his condition six weeks later. He was brought to San Francisco Feb. 27, 1914, because the headache, which had set in ten days before, persisted, and was attended with vomiting and a slow pulse. Now there was incipient but distinct papillitis in both eyes. Anosmia was found at the left nostril, and a few days later there was evidently a reduction of the sense of smell at the right also. The pulse rate was 53 on admission, and had been as low as 48. The headache, which was frontal, was intense and the patient was delirious a good deal of the time, and tossed about wildly in his pain; but there were intervals when the pain subsided and the mind was lucid, and during such an interval it was possible to test his visual fields again, roughly, but no hemianopsia of any kind could be made out. Ankle clonus could generally be provoked on the right, and occasionally on the left. From the sole of either foot a normal (flexor) as well as an abnormal (extensor) response could be elicited: a flexor response followed

on stroking the sole in the hollow of the foot, an extensor when the stimulus was applied to the skin over the heel. By the Oppenheim method either normal flexion of the big toe was obtained, or no response at all. In all other respects the result of the neurological examination was negative. The Roentgen ray showed a normal sella turcica. The blood counts and the Wassermann test of the serum yielded nothing worth noting. The hemoglobin was 75 by the Talqvist scale. It was decided not to do a lumbar puncture. There is no record of the blood pressure.

The body of the boy was not as lean as might have been expected from his thin visage, but there were no local accumulations of fat. According to the mother's statement, the child's appetite had been keen in the beginning of the disease and he had grown fat, but later it was ordinary. Puberty was not clearly developed. The testicles were not examined; there was nothing striking about the penis. The voice was that of a child.

On account of the vomiting, but little could be given by the mouth, but he continued to pass more liquid than the sum of what he drank and what he could have absorbed from the enemas. During the few days he was under observation in the city, the largest quantity of urine collected in twenty-four hours was 126 ounces; one voiding, however, was lost, and this may have amounted to a considerable quantity, for he urinated 24, 29, 30, 32 and 36 ounces at one time. The avidity with which he seized and drank whatever was offered to him showed the persistence of his polydipsia to the end.

The temperature in the mouth ranged, on February 27 and 28, between 98.6 and 99.7 F. At 11 p. m., March 1, the rectal temperature was 96.8. The oral temperature rose the next morning to 98.4 and fell in the afternoon to 97.8. On the morning of March 3, 96.1 was recorded by the mouth, being the lowest on that day; March 4 at 6 a. m. the mouth temperature was 95; the following morning it had risen to 96.8, and at 10 in the night of March 5 it had dropped to 94.4, the minimum of the whole course. By 5:30 in the morning of March 6 it was 95.5. About two hours later, while eating, the patient had a turn for the worse and soon expired.

Necropsy.—The necropsy had to be performed very hastily and incompletely, so that only the brain and the hypophysis, removed separately, could be obtained.

Nothing special was noted about the skull. The sella turcica was normal. There had been a fresh hemorrhage, no doubt the immediate cause of death. The effusion was found at the base of the brain, chiefly under the pia covering the pons and the interpeduncular space. The optic nerves were of a rather striking butter-yellow color. The anterior aspect of the hypophysis was of the same color, while the posterior was dark red. No infundibular stalk was seen.

After the brain had been hardened in formaldehyd, the corpus callosum and the fornix were removed, and the tela choroidea was reflected without any abnormal attachment being encountered. The third ventricle was then seen to be occupied by a long, dark-red tumor, into which the fatal hemorrhage had taken place. The pineal gland was very small, a thin leaflet, and might easily have been overlooked.

Microscopical Examination.—A series of sections was cut through the tumor in situ, and these were stained by the method of Weigert or that of Van Gieson. The Weigert sections, which are here reproduced, illustrate the extent and relations of the tumor, and may serve in place of much tedious description. They are ordered in the direction from nose to occiput.

Figure 1 shows the tumor in the space between the two hemispheres, near the base of the frontal lobes. In Figure 2 it is seen to occupy the whole space between the anterior commissure above and the optic nerves below, the latter just joining to form the chiasm. The growth is here in intimate connection with the dorsal surface of the united optic nerves. The optic chiasm appears next (Fig. 3), flattened and stretched over the basal surface of the tumor. In the planes next following, as the optic tracts diverge from the chiasm they

look in the sections as if they supported the tumor on either side of its ventral aspect; between these tracts the tumor protrudes, the tuber cinereum, normally situated here, having been destroyed. In this region, according to Professors Ophüls and Rusk, it is probable that the tumor originated, having sprung from one side of the infundibulum. In Figure 4, in a plane a little farther backward, the optic tracts are seen to have diverged sufficiently to leave the growth; the remnants of the tuber, through which the tumor has forced its way, seem to dangle at either side. The distention of the third ventricle, and the complete separation of the hemispheres at the base are conspicuous at this

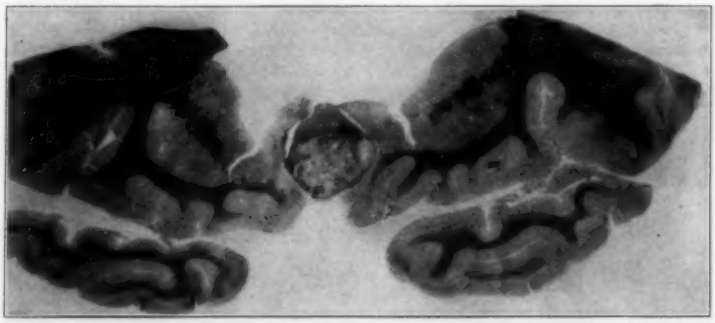

Fig. 1.—Tumor in the space between the two hemispheres, near the base of the frontal lobes.

Fig. 2.—The tumor is seen to occupy the whole space between the anterior commissure above and the optic nerves below, the latter just joining to form the chiasm. The growth is in intimate connection with the dorsal surface of the united optic nerves.

level. The lenticular nuclei, the internal capsules, the descending pillars of the fornix, the structures of the optic thalami, the gray lining of the ventricle laterally to the tumor, appear pushed aside without having suffered visible damage. The section reproduced in Figure 5 passes just anteriorly to the pons, some of the pontile tissue being included on one side, and shows the tumor occupying the interpeduncular space. The pale structures situated on either side between the tumor and the peduncle are the corpora mamillaria, elongated, flattened and widely separated by the intervening growth; a thin

membrane stretched between them forms the floor of the ventricle. The same relation between the tumor, on the one hand, and the mamillary bodies, the optic thalami and the hypothalamic region on the other, appears in Figure 6, where the section passes through the pons. The growth is shown, not far from its posterior end, lying in the ventricle, in Figure 7, which illustrates a section passing through thalami, red nuclei and pons.

The tumor did not extend to the posterior commissure and the pineal body. The aqueduct of Sylvius was not dilated. No lesion was found in the medulla oblongata or fourth ventricle.

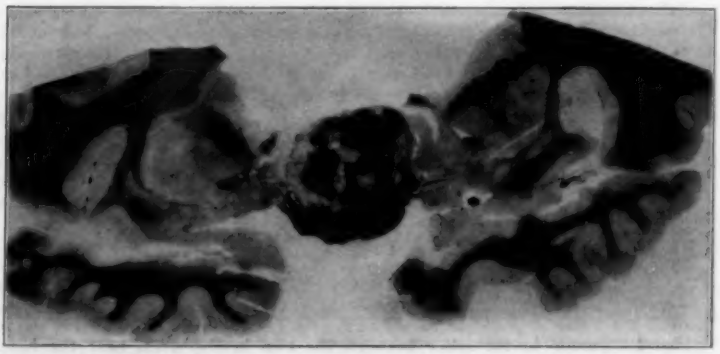

Fig. 3.—The optic chiasm appears flattened and stretched over the basal surface of the tumor.

Fig. 4.—The optic tracts, which in a more frontal plane seemed to prop the tumor, have diverged from it. Note protrusion of tumor through tuber cinereum.

The dark areas in the tumor denote hemorrhage. The growth is very rich in cells, which show a distinct alveolar arrangement. There is considerable connective tissue. It has not been found possible to establish with certainty the nature of the tumor; the stronger inclination has been to consider it to be of an epithelial character. As already indicated, the infundibulum is held to be the most probable place of origin. Sections of the tela choroidea did not reveal tumor cells there.

The hypophysis: No remainder of the infundibular stalk or of the pars

nervosa could be identified. The organ consists of pars anterior and remnants of the pars intermedia. In stained sections through the region of the pars intermedia there is visible to the naked eye an area somewhat conical in shape, about 1 mm. wide and nearly 2 mm. deep, penetrating from the fibrous covering of the hypophysis into the organ; this area is conspicuous from the intensity with which it has taken the hematoxylin. The normal tissue is replaced there by a fairly cellular connective tissue, in which there are large cells with very large, very deeply staining nuclei. These cells are either polygonal or somewhat elongated. There is quite a heavy infiltration with lymphocytes throughout this tissue. Between this growth and the tissue of the anterior lobe the boundary is partly fairly well marked, but along a line it becomes ill-defined, and here the large cells of the tumor and the smaller cells of the glandular part of the hypophysis are in places intimately mixed. At the apex of the wedge of tumor tissue cysts and follicles of the pars intermedia are to be seen in fair number; they contain oxyphil colloid. The follicles are lined with clear nuclei.

Fig. 5.—See legend of Figure 4. This section passes just anteriorly to the pons, some of the pontile tissue being included on one side. The tumor occupies the interpeduncular space. See text for further description.

In anteroposterior sections beyond the region of the pars intermedia the tumor is superficial, lying directly beneath the infiltrated capsule of the hypophysis, in a shallow depression of the anterior lobe, and for the most part well defined against the hypophysis, but in one region intimately connected with it. The pars anterior contains eosinophil and chromophobe cells in the usual proportions, but is deficient in basophil cells. The fibrous capsule of the hypophysis, where it covers the tumor, contains blood resulting from a recent hemorrhage.

Pineal Body: This structure had the usual shape of a cone. As has been remarked, it was quite small; its length did not exceed 4 mm.; its greatest thickness was a trifle more than a millimeter and a half; its width cannot

be given in figures, but it was proportionate to the other dimensions. It had been cut off close to the habenular commissure and sagittal sections were made.

Under the microscope the most conspicuous object is an accumulation of brain sand, situated in about the middle of the organ. Between this and the apex of the conical body there is a great scarcity of cells, the meshes of the fibrous framework being for the most part empty. The fibrous tissue is hardly abnormally developed here, but there is rather more of it than in the region toward the base of the gland. Among the deposits of sand there is a group of lymphoid cells around a vessel which itself shows no changes. In the area between the concretions and the base, the cells are present in considerably larger number than toward the apex, but still are relatively sparse. The large round nuclei of the characteristic cells are chiefly of the light variety, with

Fig. 6.—Section passing through the pons. See text.

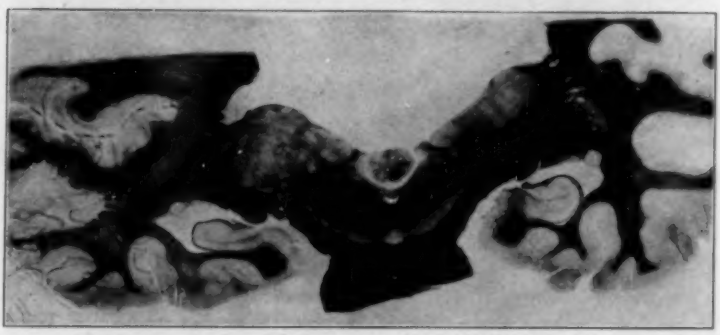

Fig. 7.—Tumor near its posterior end in third ventricle. The band of fibers on either side of the tumor is the fasciculus retroflexus of Meynert.

fine granules; sometimes the granules are arranged like a wreath around the periphery of the nucleus. There are also very pale, shadowy nuclei. Nuclei with the coarser granules are deficient. In a large transverse section through the brain stem, stained to show the medullary sheaths, a group of cells, considerable in number, is seen close to the posterior commissure; they evidently belong to the pineal body, but the method of staining precluded differentiation of them.

The pineal body, accordingly, is atrophied, and the disappearance of cellular elements is more marked in the direction of the apex than toward the base.

DISCUSSION

Diabetes insipidus, arrest of growth and low temperature were observed in combination before there was a knowledge of their relation to the hypophysis or its neighborhood. The low temperature was ascribed to "the loss of heat resulting from the increased elimination of water and the frequent ingestion of cold fluids."[1] In the case before us the variations of temperature are known only as they occurred in the last days of life. There was no relation at that time between imbibition and elimination of fluids, on the one hand, and the degree of temperature on the other; rather did the regulation of temperature appear to become disturbed in accordance with the increase of congestion in, and pressure by, the tumor, and the lowest temperature, recorded on the last night might have been regarded as an omen of the final issue, which occurred the next morning.

To the units of the symptom complex mentioned should be added the tolerance of drugs, and perhaps intolerance of them. Like the lowering of the temperature, the tolerance of large doses was attributed to the activity of the urinary secretion. Thus Guinon[1] wrote in 1890:

> One phenomenon which is associated with the activity of the urinary secretion is the immunity against alcoholic drinks and drugs. Extract of valerian in large doses, ergotin in grammes continued for several days, antipyrin in doses of a drachm and a half administered regularly, produce no bad effect on these children, because the active element of the drug is eliminated by the lavage of the tissues.

This is an obvious explanation for increased tolerance; but it would not account for the "exaggerated susceptibility" which has been asserted in regard to alcohol as well as to other substances.[2] Few records of original observations on diabetes insipidus in which mention is made of the effect of drugs have come to my knowledge from the older literature, and none from that of recent years; and judgment concerning the earlier cases is rendered difficult by the uncertainty of their pathology. It is improbable that all the polyurias described as diabetes insipidus have an identical pathology. For instance, what is one to assume to be the lesion in a man[3] 58 years of age, who was admitted with what was erroneously considered a classical alcoholic delirium, who had had polydipsia and polyuria for thirty-one years? In the history which was delivered with the patient it was stated that he tolerated alcohol badly, but the patient denied, on recovery from the

1. Guinon, L. J.: Urinary Neuroses of Childhood, Wood's Medical and Surgical Monographs, 1890, p. 715.

2. Ralfe and Bradford: Chapter on Diabetes Insipidus in Allbutt and Rolleston's Syst. Med., 1907, **3**, 212. Tyson's Practice of Medicine, Ed. 5, 1910.

3. Julius Wagner: Vermeintliches Alkohol-Delirium, Diabetes insipidus. Salicylsäurevergiftung, Wien. klin. Wchnschr., 1888, **1**, 778.

delirium, that he had been drinking, and the diagnosis of the etiology was regarded as invalidated by the discovery of the polyuria of 7,000 to 9,000 c.c. with a specific gravity of 1.003 to 1.005 as "it was well known that patients with diabetes insipidus have a remarkably great tolerance for alcoholic drink." The delirium was thereupon ascribed to sodium salicylate, of which the patient had taken rather large doses; this had had the effect of greatly reducing the excretion of urine. The inference seemed to be that insufficient elimination had thus led to intoxication. But in a case described by Trousseau,[4] apparently the *observatio princeps* in this matter, there was associated with an amazing endurance of alcohol an extraordinary sensitiveness to certain other drugs. The patient in this instance was a youth of 20, whose polydipsia and polyphagia had begun at 16. "He stated that from the beginning he had acquired so great an immunity as to be able to drink large quantities of stimulants without feeling the slightest symptoms of being drunk. On several occasions he took on a wager 20 liters of wine, gaining his wager without producing any effect on the nervous system." At a time when the patient was drinking "33 liters in the 24 hours, and passing from 37 to 43 litres in the same space of time," Trousseau "tried belladonna in doses of one centigramme; and — strange to tell! — this man, who could drink 20 litres and a litre of alcohol of sp. gr. 0.835 (*90 degrés centésimaux*[5]) without being intoxicated, experienced violent effects from this minute dose of belladonna; and each time it was repeated similar results were produced. I then had recourse to strychnia." But strychnin was likewise not tolerated.

This was undoubtedly a severe case of primary polyuria, not a primary polydipsia of purely functional, or psychic, character. The patient was one of those who "seized the chamber-pot, and drank the contents to the last drop" while "enduring an almost total withdrawal of drinks." There had been a small quantity of sugar in the urine at times at the beginning of the malady. "Convulsive phenomena" and "failing of sight" are mentioned. All of which circumstances concur to fortify the impression of a pituitary or infundibular diabetes insipidus.

In the course of extensive, but still far from exhaustive, study of the literature I have not come on any case in which the tolerance of drugs had been observed and the cause of the polyuria had been certified by necropsy. The case with which this paper deals demonstrates that in a polyuria resulting from infundibular disease, the brain may

4. Trousseau: Lectures on Clinical Medicine, New Sydenham Society's Translation, 1870, **3**, 528.

5. The translator explains that a liter of alcohol *à 90 degrés centésimaux* is rather more than a quart of alcohol at specific gravity 0.835, or 57⅛ overproof, which is a trifle stronger than the rectified spirit of the British Pharmacopeia.

not be affected by alcoholic draughts to which immaturity, a previous injury to the skull, and the presence of a large vascular tumor might be supposed to render it peculiarly sensitive. On what this immunity depends need not be left to speculation, but should be determined by investigation on appropriate human or experimental material.[6]

Abundant evidence has been collected at necropsy, as well as by experimental work, to establish the dependence of polyuria on disease localized in the region in which the tumor was found in the present case. But it has been found difficult to explain the process by which the abnormal diuresis is produced, and the very organ involved is subject to dispute. It is probably no mere coincidence that the neurohypophysis should furnish an extract with diuretic properties, and that disease in, and about, the neurohypophysis should be so often associated with diabetes insipidus. Yet the theory that the polyuria was due to an excess of diuretic substance has but little support from observations on man. Indeed, this theory yielded under the influence of postmortem data to the assumption that there was a deficiency of the normal restraint on diuresis, and this assumption seemed to be confirmed by the observation that pituitary extracts might diminish the amount of urine in normal persons as well as in patients with diabetes insipidus. One should, however, be cautious in concluding that these extracts are specific antidiuretics. The literature[7] of a little over thirty years ago contains accounts of remarkable effects of ergot in diabetes insipidus, and that, too, in some cases which seem from the concomitant optic atrophy or headaches to have been of intracranial origin. The therapeutic results reported there excel those which seem to be obtained by the use of pituitary extracts.

Excessive function of the pars intermedia or pars posterior, as well as diminished function of those parts, having failed to account for the polyuria, the notion of "dysfunction" has been introduced; and finally there are pathologists[8] and experimental investigators who reject hypophyseal pathology altogether from consideration in relation to polyuria. Thus, Camus and Roussy,[9] who have experimented very diligently on

6. Ralfe and Bradford (Allbutt and Rolleston's Syst. Med., 1907, **3,** 216) mention the great fall in the amount of urine, often to normal, on the accession of pyrexia. Long ago Todd asserted that in pyrexia large doses of alcohol were borne without intoxication, lessened tolerance indicating the return of the temperature to normal. These statements conjoined might be made to support the view that tolerance of alcohol is independent of diuresis. In diabetes insipidus one would expect the tolerance of alcohol to be associated with a subnormal temperature.

7. DaCosta, J. M.: Ergot Treatment of Diabetes Insipidus, Med. News, Jan. 7, 1882, p. 5.

8. Erdheim: Ueber Hypophysenganggeschwülste, Sitzungsb. d. Wien, Akad. d. Wissensch., 1904, **113,** 537.

9. Camus and Roussy: Diabète insipide et polyurie dite hypophysaire, Presse méd., July 8, 1914, No. 54.

the production of polyuria, turn from the posterior lobe of the hypophysis as a mere "atrophied nervous fragment" and seek higher, at the base of the brain, in the gray matter of the third ventricle, the centers which regulate the assimilation of carbohydrates and the water content of the organism. According to their views, the presence of the tumor in the "opto-peduncular space" in the case I am reporting would amply account for the polyuria, for they could produce a copious and durable polyuria by a lesion in that region, even in animals which had previously been deprived of the hypophysis.

A brief survey of necropsy data will illustrate the difficulty of discerning the essential factor in the provocation of diabetes insipidus. The problem is akin to that of accounting for the relation between the hypophysis and "adipositas hypogenitalis," which Bernhard Fischer[10] has discussed, coming to the conclusion that this state is shown by postmortem examination in the human being to be generally the result of purely mechanical injury, by pressure, to the pars nervosa. He finds no explanation for the exceptions, that is, for the cases in which the anatomical conditions are given, but the dystrophia adiposogenitalis was absent. The pathological-anatomical conditions being about the same as those found associated with diabetes insipidus, it is striking that polyuria is mentioned in only four out of thirty-one cases of adiposity with tumors in the hypophysial region, which were tabulated by Strada.[11]

(*a*). Any kind of pathological process, tuberculosis, syphilis, actinomycosis,[12] primary or metastatic tumors, or trauma, established in or near the hypophysis may provoke a polyuria. It has been supposed that the effect was the result of irritation of the pars intermedia. Von Gierke[13] got the impression of "chronic inflammation of the pars posterior with hyperplastic proliferation of the cells of the pars intermedia" in a somewhat obscure case with moderate polyuria. Schmorl[14] found in two cases of myxedema a not inconsiderable hyperplasia of the hypophysis, and especially of the pars intermedia, but does not mention polyuria.

(*b*). Metastatic carcinoma has in a number of instances been confined to the pars nervosa, leaving the pars intermedia intact. In a case reported by Götzl and Erdheim[15] there was a large tumor in the third ventricle, the relations of which to the floor of the ventricle were not

10. Fischer, B.: Hypophysis und Adipositas hypogenitalis, Frankfurter Ztschr. f. Pathol., 1912, **11,** 145.

11. Strada: Virchows Arch. f. path. Anat., 1911, **203,** 1.

12. Belkowski: Actinomycose de la base du crâne et des méninges. Diabète insipide, Rev. de méd., 1911, **31,** 420.

13. Von Gierke: Verhandl. d. deutsch. path. Gesellsch., 1914, **17,** 200.

14. Schmorl: Verhandl. d. deutsch. path. Gesellsch., 1914, **17,** 231.

15. Götzl and Erdheim: Ztschr. f. Heilk., 1905, **26,** 372.

unlike what has been described in my case, but the hypophysis had undergone only slight compression, its structure, however, being considered unchanged, apart from large foci of small cells in the posterior lobe. Diabetes insipidus had persisted for two years. Irritation of the pars intermedia, or obstruction to channels leading from the pars nervosa, was a possible effect of the tumor.

(*c*). But the pars intermedia, and indeed the whole hypophysis, may be destroyed by new growth, and yet the polyuria may persist to the end. It would require the extraction of a diuretic substance from such a tumor to support a chemical hypothesis, unless it could be made plausible that some other organ was stimulated to excessive vicarious production of a diuretic substance.

(*d*). It appears to be an obstructive or destructive process affecting the pars nervosa, not an irritative process affecting the pars intermedia, that excites the polyuria.

(*e*). But the polyuria and polydipsia have been known to subside after having lasted for weeks, where extensive destruction of the hypophysis by cancer had taken place,[16] and after having lasted for years (in Götzl and Erdheim's case) when the hypophysis was only slightly affected.

(*f*). There is a considerable body of observations concerning tumors and other morbid processes affecting the hypophysis which failed to produce polyuria. Simmonds,[17] for instance, found cancerous metastases without polyuria as well as with it, and he has also reported very advanced atrophy of the hypophysis in all its parts without the occurrence of diabetes insipidus.

Seeking for the cause of the effect in the most limited lesion, we may conclude that disease affecting the neurohypophysis may provoke polyuria (and until now it has appeared to be the most frequent provocative of it); but the effect need not be permanent, and it need not occur at all. This irregularity in the concurrence of disease of the neurohypophysis and diabetes insipidus may be a consequence of the fact that the neurohypophysis is not the only region from which polyuria and polydipsia may be elicited by disease.

While the medulla oblongata figures infrequently at present in the pathology of diabetes insipidus, growths in the pineal body have become associated with this disorder. Abnormalities of growth and sexual development having been referred to pineal tumors, a likeness

16. Simmonds: Zur Pathologie der Hypophysis, Verhandl. d. deutsch. path. Gesellsch., 1914, **17**, 208.

17. Simmonds: Ueber sekundäre Geschwülste des Hirnanhangs und ihre Beziehungen zum Diabetes insipidus, München. med. Wchnschr., 1914, No. 4, p. 180; also, Ueber Hypophysisschwund mit tödlichem Ausgang, Deutsch. med. Wchnschr., 1914, No. 7, p. 322.

of the symptomatology of these growths to that of hypophyseal tumors has appeared, and it is enhanced by the inclusion of diabetes insipidus among the disturbances that may arise from either. Also a substance with diuretic effect has been extracted from the pineal body (of the sheep).

From the publications on polyuria in connection with pineal tumors, it will suffice to cite, first, a report by v. Hösslin[18] on a boy aged 9, who for several months drank and passed 20 to 24 quarts a day, the urine being of low specific gravity. Several weeks before death the diabetes insipidus ceased: an occurrence such as has been mentioned above as observed in hypophysial diabetes insipidus. A sarcoma of the pineal body was found in this case. Then there is the record of another boy[19] in whom the pineal tumor had been diagnosed during life: he had unquenchable thirst with enormous polyuria. In the reports of these cases which I have been able to see, the condition of the hypophysis is not mentioned; but it is mentioned in a third case, of which v. Gierke[20] gives an account. The patient was a victim, at 72 years, of metastatic carcinoma in the bones, kidneys, dura, and in the pineal body; six weeks before death an increase of the urine to 4 or 5 liters had occurred, and it continued until the end. At night water was drunk "by the can." Despite the cancer the corpse was adipose. There were found in the hypophysis only traces of a minute hemorrhage at the posterior border of the anterior lobe, which was not held responsible for the insipid polyuria. Apart from a slight change in the thyroid, the other glands of internal secretion were normal, so that v. Gierke thought only the pineal disease deserved consideration with respect to the polyuria.

What the relation of the pineal atrophy in my case is to the tumor in the infundibulum I am not able to determine by reference to other cases. Simmonds found no changes in the pineal body in his material of diseased hypophyses. Bartlett[21] describes it as enlarged in a case of adenoma of the anterior lobe of the hypophysis with destruction of the posterior.

SUMMARY

1. An account is given of a boy who died at 14 years after having had diabetes insipidus continuously for about five years.

2. Signs of a tumor appeared only about two weeks before death; whence the admonition against assuming the "functional" nature of a

18. Von Hösslin: München. med. Wchnschr., 1896, No. 13, p. 292.
19. Hijmanns: München. med. Wchnschr., 1913, No. 38, p. 2140.
20. Von Gierke: Hypophysis und Epiphysis bei Diabetes insipidus, Verhandl. d. deutsch. path. Gesellsch., 1914, **17**, 200.
21. Bartlett, F. K.: A Case of Acromegaly and Polyglandular Syndrome, with Special Reference to the Pineal Gland, THE ARCHIVES INT. MED., 1913, **12**, 201.

diabetes insipidus even after some years have elapsed without definite tumor symptoms.

3. A craving for alcoholic drink and unusual tolerance of it in diabetes insipidus are recorded. There is a need for more observations on the effects of drugs in this disorder.

4. A tumor occupying the region of the infundibulum, extending forward through the lamina terminalis, between the frontal lobes, and backward into the third ventricle and destroying the neurohypophysis and most of the pars intermedia, accounted for the diabetes insipidus.

5. There was also atrophy of the pineal body.

6. A survey of the literature confirms the view that a tumor causing diabetes insipidus is commonly situated in, or near, the neurohypophysis, but occasionally in the pineal body. It does not appear from clinico-pathological observations that it is overproduction of a diuretic substance that causes diabetes insipidus.

THE REACTIVATED THYMUS*

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"Persistent thymus" has been a term in the literature, though a term about which much skepticism has gathered. But it is gradually becoming established as a fact that the thymic parenchyma persists throughout life, and that this parenchyma may become reactivated to the extent that its secretion influences the endocrine equilibrium. The causes of such a reactivation are not known. Studies of the histologic picture afforded by such a reactivation are rare; hence the following data from a case that for want of a better term may be called thymic myasthenia.

REPORT OF CASE

History.—G. N., a farmer, first seen at the age of 19, had been suffering for two years from shortness of breath. He had measles as a child; no other diseases except a headache about three to five times a year since 10 years of age. The patient vomited at the end of each attack. Formerly he was the "fattest" one in the family, but was now thin. Sleep was poor on account of a "stretching of the diaphragm"—evidently a feeling of constriction.

Examination.—The examination showed a short but well-proportioned young man, with no external evidence of disease. The stomach was dilated, reaching to the navel. Diastolic blood pressure was 95, systolic 130. The urine was negative, except for increased indican. The white blood cells numbered 13,600, polymorphonuclear cells 65 per cent., and large lymphocytes 33. Gastric lavage and intestinal cleansing had no effect on the subjective symptoms. Four ounces of sugar caused no glycosuria. Epinephrin (adrenalin), 1 c.c. of 1:3,200 solution, raised the temperature in twenty minutes from 98.6 to 99 F., the pulse remaining at 84. The temperature was labile and easily raised by emotional and physical excitement. The condition was not affected by the administration of thyroid extract 1 grain three times a day for one month.

Examination by surgeon (Dr. Binnie) was negative; by neurologist (Dr. Skoog), negative; roentgenogram of spine (Dr. Skinner), negative.

Findings.—The only important physical finding was increased submanubrial dulness, the outlines of which are shown in the accompanying photograph (Fig. 1). The roentgenogram of the chest (Fig. 2) shows an enlarged shadow in this area.

The manubrium was resected (Dr. Binnie) and some pieces of thymus removed. The microscopic study of these (Figs. 3 and 4) shows the epithelialization described by Matti and Klose as characteristic of the reactivation of the adult thymus.

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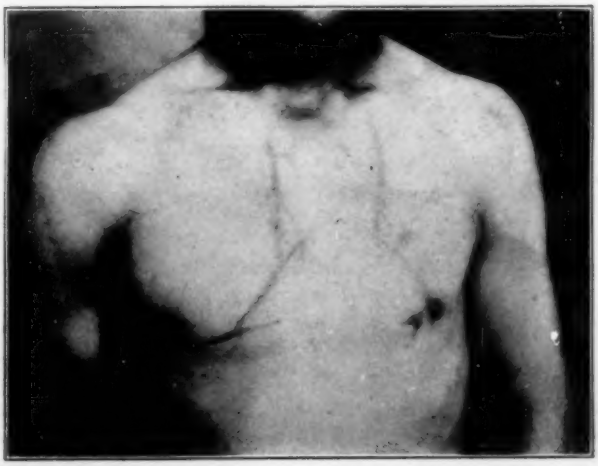

Fig. 1.—Showing the area of thymus dulness.

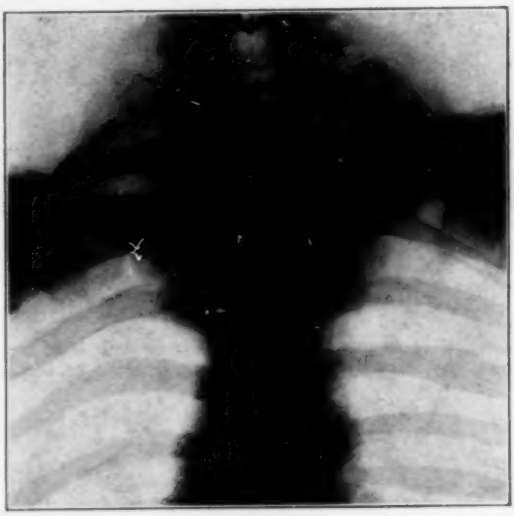

Fig. 2.—Roentgenogram showing the increased shadow in the thymic area.

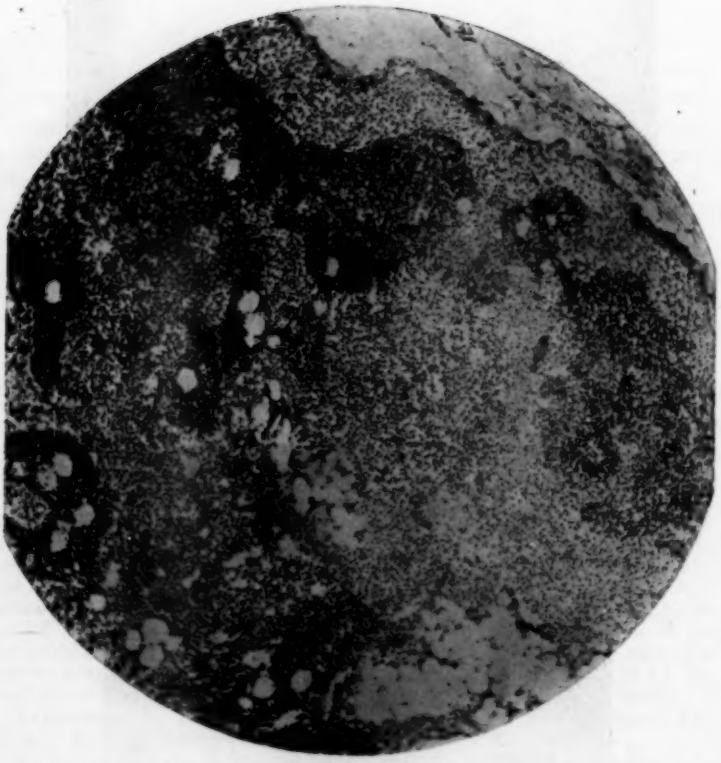

Fig. 3.—Section of thymic tissue under low power. Note the increase in the light areas, the Hassall corpuscles and the inroads of the vacuolization.

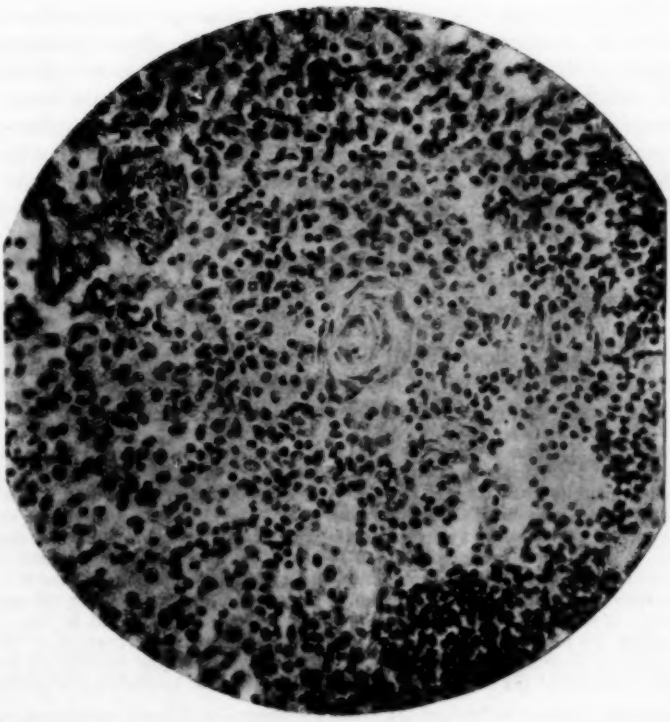

Fig. 4.—Higher magnification of the central portion of the low power field shown in Figure 3.

A STUDY OF THE DIASTATIC ACTIVITY OF URINE AND FECES WITH SPECIAL REFERENCE TO DISEASES OF THE PANCREAS *

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Any procedure which will enable the clinician to determine the functional condition of an organ is most desirable. From the investigations of Wohlgemuth,[1] Wynhausen[2] and Noguchi[3] on the quantitative estimation of diastatic activity in the blood, urine and feces, it seemed as though a method had been found which would show changes in the functional state of the pancreas. The results reported by these authors were obtained, not only in experimental pancreatic lesions in animals, but in pancreatic disease in man. Their work led to the present study.

TECHNIC

The method employed to estimate diastatic activity was one devised by Wohlgemuth.[4] The principle of the method is the conversion of starch into erythrodextrin, as determined by the iodin reaction. Wohlgemuth's modification of his method has been used in our investigation because of the short time in which it can be performed.

Varying amounts of urine or a fecal suspension, decreasing by arithmetical progression from 1 c.c. to 0.1 c.c., are added to a series of ten test tubes. If a smaller quantity than 0.1 c.c. of urine is required, the urine is diluted ten or twenty times with distilled water. The test is then repeated, using the same amounts of the diluted as of the undiluted urine. To each test tube are added 2 c.c. of a 0.1 per cent. soluble starch solution. The tubes are then shaken and placed in the water bath or incubator at 38 C. for thirty minutes. At the end of that time fiftieth-normal iodin solution is added drop by drop to each tube until a color is produced. The end-reaction is considered to be present in that tube in which the blue color of starch is replaced by the reddish-brown of erythrodextrin. The units expressing the diastatic activity are the number of cubic centimeters of 0.1 per cent. starch solution, which will be converted into erythrodextrin by 1 c.c. of urine and the symbol employed is $D^{30'}_{38°}$. In working with

* Submitted for publication Oct. 23, 1916.

* From the Laboratories of Medicine of Harvard University, Boston, and of the State University of Iowa, Iowa City, Iowa.

1. Wohlgemuth: Beitrag zur funktionellen Diagnostik des Pankreas, Berl. klin. Wchnschr., 1910, **47,** 92.

2. Wynhausen: Zur Funktionsprüfung des Pankreas, Berl. klin. Wchnschr., 1910, **47,** 478; 1909, **46,** 1406.

3. Noguchi: Ueber d. Fermentdiagnose bei Pankreasverletzung, Arch. f. klin. Chir., 1912, **98,** 545.

4. Wohlgemuth: Ueber eine neue Methode zur quantitativen Bestimmung des diastischen Ferments, Biochem. Ztschr., 1908, **9,** 1. Wohlgemuth and Noguchi: Experimentelle Beiträge zur Diagnostik der subcutan Pankreasverletzungen, Berl. klin. Wchnschr., 1910, **47,** 478; 1912, **49,** 1069.

feces a filtered 10 per cent. extract in 1:1,000 sodium carbonate was used. The maximum quantity of the fecal extract was 2 c.c. instead of 1 c.c. as with urine, otherwise the technic was the same.

The original method of Wohlgemuth differs from the above-described modification in that 5 c.c. of a 1 per cent. starch solution are employed and the incubation time is twenty-four hours.

DIASTASE IN THE URINE OF PERSONS WITH NORMAL PANCREAS

Wynhausen,[5] using Wohlgemuth's original method, studied the diastatic activity in the urines of 238 patients in whom the pancreas was not diseased. He considered the normal limits to lie between 50 and 156 units in spite of the fact that the urines in six cases contained from 200 to 500 units, and in sixty-nine, less than 50 units. The majority of subsequent writers have accepted these limits, fixed arbitrarily by Wynhausen, as representing the normal.

Benczur[6] reported from 10 to 500 diastase units in the urines of forty-eight patients in whom the "pancreas was normal." We have collected from the literature 459 cases[7] without pancreatic disease, in which the diastatic activity of the urine is expressed in the units of Wohlgemuth's original method. The urines of 171, or 37 per cent., of these patients contained less than 50 diastase units. The urines of fifty-eight, or 13 per cent., of these patients contained from 100 to 200 diastase units, in eighteen cases, or 4 per cent., from 200 to 500 units, and in three cases, or 0.7 per cent., reported by Wynhausen, "more than 500 units." Cases of nephritis and of diabetes mellitus have been included in this list. The statement is frequently found that the diastatic activity of the urine is low in these two diseases. An analysis of the reported figures shows variations from "less" than 20 to "more" than 500 diastase units. Brown[8] using a modification of Wohlgemuth's technic, reported from 1,500 to 12,000 diastase units in the urines of forty-four normal adults. It should be noted that Brown's results are

5. Wynhausen: Quantitative Diastasebestimmungen in Harn, besonders ihre Beziehungen zur Nephritis und zum Diabetes Mellitus, Berl. klin. Wchnschr., 1910, **47,** 2107.

6. Benczur: Beitrag zur klinischen Verwertbarkeit der Diastasemenge in Blutserum und Urin, Wien. klin. Wchnschr., 1910, **23,** 890.

7. Wynhausen: Zur Funktionsprüfung des Pankreas, Berl. klin. Wchnschr., 1910, **47,** 478; 1909, **47,** 1406; Quantitative Diastasebestimmung in Harn, besonders ihre Beziehungen zur Nephritis und zum Diabetes Mellitus, Berl. klin. Wchnschr., 1910, **47,** 2107; Benczur: Beitrag zur klinischen Verwertbarkeit der Diastasemenge in Blutserum und Urin, Wien. klin. Wchnschr., 1910, **23,** 890. Marino: Ueber die diagnostische Bedeutung der Diastaseausschiedung im Harn, Deutsch. Arch. f. klin. Med., 1911, **103,** 325. Lindemann: Zur diagnostischen Bedeutung des Diastasegehaltes in Urin und Stuhl, Ztschr. f. klin. Med., 1912, **75,** 58.

8. Brown, T. R.: The Normal Amount of Diastatic Ferment in the Urine and Feces and Its Variation in Diseases of the Pancreas, Tr. Assn. Am. Phys., 1914, **29,** 547.

not comparable with the others given, as he determined the diastatic activity not for one cubic centimeter but for the entire twenty-four-hour amount of urine.

For his modified method Wohlgemuth[9] reports 64 units to be the maximum normal limit found for the diastase of the urine. But he does not state on what observations this conclusion was based. We have collected 216 cases[10] without evidences of pancreatic disease, in which the diastatic activity of the urine was estimated by Wohlgemuth's modified method. Variations from none to 66.6 diastase units were present in the urines of 208 of these patients; 100 units were present in the urines of seven patients, and 400 in one instance.[11]

TABLE 1.—DIASTATIC ACTIVITY IN THE URINE OF NORMAL ADULTS

Diastase Units in 1 C.c. Urine	Number of Cases
4	2
8	2
20	5
40	12
80	1

We have studied the diastatic activity of the urine in healthy adults and in hospital patients. Estimations of the diastatic activity were made in the urines of twenty-two healthy male adults, obtained through the courtesy of Dr. J. A. Beer of Columbus, Ohio. All but one of these urines contained less than the maximum limit of 64 units reported by Wohlgemuth.[9]

The diastatic activity of the urine was studied in 108 hospital patients. These patients were without evidences of pancreatic disease

9. Wohlgemuth and Noguchi: Experimentelle Beiträge zur Diagnostik subcutan Pankreasverletzungen, Berl. klin. Wchnschr., 1912, **49,** 1069.

10. Rosenthal: Zur Frage der Auschiedung von diastischen Ferment im Urin, Deutsch. med. Wchnschr., 1911, **37,** 923. Geyelin: A Clinical Study of Amylase in the Urine, THE ARCHIVES INT. MED., 1914, **13,** 96. Rowntree, Geraghty and Marshall: A Study of the Comparative Value of Functional Tests in the Surgical Diseases of the Kidney Secondary to Obstruction in the Lower Urinary Tract, Surg., Gynec. and Obst., 1914, **18,** 201. Corbett: The Quantitative Estimation of Amylolytic Ferments in the Urine as a Measure of Certain Pathological Conditions, Quart. Jour. Med., 1912-1913, **6,** 351. Stocks, P.: The Quantitative Determination of Amylase in Blood Serum and Urine as an Aid to Diagnosis, Quart. Jour. Med., 1916, **9,** 216. Neumann: Das diastatische Ferment des Urins, Deutsch. Arch. f. klin. Med., 1913, **111,** 164. Wohlgemuth: Beitrag zum Verhalten der Diastase im Urin, Biochem. Ztschr., 1909, **21,** 432.

11. Corbett: The Quantitative Estimation of Amylolytic Ferments in the Urine as a Measure of Certain Pathological Conditions, Quart. Jour. Med., 1912-1913, **6,** 351.

or diabetes mellitus. Two cases of chronic nephritis showed 4 and 20 diastase units in the urines. In twenty-three of these cases the twenty-four-hour urines were examined; the remainder were morning specimens.

In these cases the degree of diastatic activity in the urines varied from 2 to 200 units. The same limits of variations, 2 to 200 units, were found in the twenty-four-hour urines of twenty-three patients. Seven of these (sciatica, salpingitis, secondary syphilis, hysteria, arthritis deformans) showed values of 200 units, and a case of chronic appendicitis, 100 units. Concentration of the urines played no rôle, since the quantities of urine containing the largest number of diastase units were as great as those urines containing the least number.

TABLE 2.—DIASTATIC ACTIVITY IN URINE OF PATIENTS WITH NORMAL PANCREAS

Diastase Units in 1 C.c. Urine	Number of Examinations	Number of Cases
2 to 8	36	33
20 to 40	63	63
50 to 70	6	5
100 to 200	22	7

It is evident from our own observations as well as those reported by others that the diastatic activity in the urine of patients with normal pancreas varies within wide limits; in fact, from a great diastatic activity to one so small as not to be demonstrable by this test. Five and four-tenths per cent. of our patients and 4 per cent. of those reported by Corbett[11] and Stocks[12] showed from 100 to 400 diastase units in the urine. These are figures well above Wohlgemuth's[9] maximum normal limit of 64 units. These variations correspond to those obtained by Wohlgemuth's original method, which showed 37 per cent. below the minimum normal limit as fixed by Wynhausen[5] and 4.7 per cent. well above his maximum normal limit. As in thirty-seven of our patients (24.4 per cent.) with normal pancreas the diastatic activity of the urine ranged from 2 to 8 units, and in four reported by Corbett[11] none was present in certain examinations, a low value cannot be regarded as evidence of pancreatic disease.

THE URINE IN MALIGNANT DISEASE OF THE PANCREAS

In the following eight cases the diastatic activity of the urine was higher than the usually accepted normal limits. Wohlgemuth,[1] using his original method, reported studies on the diastatic activity in the

12. Stocks, P.: The Quantitative Determination of Amylase in Blood Serum and Urine as an Aid to Diagnosis, Quart. Jour. Med., 1916, **9**, 216.

urines of two women in whom there was "total or at least partial occlusion of the pancreatic ducts." The urine in one case contained 625 diastase units. The "different portions" of urine in the second case gave values of 625, 1,250, and then 156 to 100 units. Six hundred and twenty-five and 1,250 units in the urines of these two patients are above the maximum of 500 units found in patients without disease of the pancreas. The clinical and pathologic findings on which the diagnoses in these cases were based are not given. Whether the "different portions" of urine were collected during one or several days is not stated. No explanation is offered for the variations in the number of diastase units.

Marino,[13] using Wohlgemuth's original method, reported 200 diastase units in the urine of a patient with carcinoma of the pancreas, and Wynhausen, 200 and 300 units in two cases. Benczur[9] reported 250 diastase units in the urine of a patient with cancerous occlusion of the pancreatic duct. The diagnoses in these cases were confirmed by operation or necropsy. Using Wohlgemuth's modified method, Corbett[11] reported 50 to 400 diastase units in the urine from a patient with pancreatic carcinoma who came to operation. One hundred units were present in the urine of a patient reported by Stocks[12] in which case the diagnosis was not confirmed by either operation or necropsy.

In twelve cases of malignant disease of the pancreas the diastatic activity in the urine was "normal" or decreased. Brown[8] reported a case of advanced carcinoma of the pancreas in which the patient's urine contained 333 diastase units (1,500 to 12,000 units regarded as normal by his method). Using Wohlgemuth's modified method, Humphrey[14] found 50 diastase units in a case of "either cancer or chronic inflammation of the pancreas." Stocks[12] reported from 12 to 28 diastase units in the urines in four cases of malignant disease of the pancreas in which the diagnoses were confirmed by necropsy. In six cases in which a clinical diagnosis of carcinoma of the pancreas was made, but unsupported by operation or necropsy, the urines contained from 5 to 66 diastase units.

We have estimated the diastatic activity in the urines in three cases of carcinoma of the pancreas in which the diagnoses were confirmed by operation or necropsy. The stools in all the cases were very fatty. The patients were jaundiced. In two of these cases single examinations of the urine were made. The urine of one patient contained 4 diastase units and of the other, 40 units. Two samples of the remain-

13. Marino: Ueber die diagnostische Bedeutung der Diastaseausschiedung im Harn, Deutsch. Arch. f. klin. Med., 1911, **103,** 325.

14. Humphrey: The Urinary Diastase Test and Loewi's Reaction in Pancreatic Lesions, Brit. Med. Jour., 1914, **1,** 1229.

ing patient were collected on different days and contained 4 and 8 diastase units.

To recapitulate: Seven cases with the unconfirmed diagnosis of malignant disease of the pancreas and ten confirmed by operation or necropsy have been collected from the literature. To the latter our three cases are to be added, making thirteen in all. Five of these thirteen patients showed an increase in the diastatic activity of the urine above the usually accepted normal limits. In one of these the diastase power of the urine was at times normal. In none of these twenty cases did the diastatic activity exceed that observed in patients without pancreatic disease. In two of our three cases and one reported by Brown, low values were obtained. But as we have shown, a low value may also be found when the pancreas is normal. Hence no importance can be attached to a low diastatic activity of the urine in the diagnosis of cancer of the pancreas.

THE URINE IN CHRONIC PANCREATITIS

Hirschberg,[15] using Wohlgemuth's original method, reported 25 to 100 diastase units in the urines in a case of chronic interstitial pancreatitis. Marino[13] found no diastatic power in the urine in a case of atrophy of the pancreas and 6 units in the urine in a case of slight pancreatic atrophy. The diagnoses in these three cases were verified by necropsy. Ehrmann and Kruspe[16] investigated a case in which the stools were large, fatty and azotorrheic. Metabolism studies showed absorption of 66 per cent. of the fat of the food. The patient was jaundiced. At operation the pancreas was found to be very hard throughout and the ductus choledochus pressed on by the head of the pancreas. Jaundice disappeared after the operation, but the character of the stools remained unchanged. Before operation the twenty-four-hour urine contained 80, 12.5, and 25 diastase units. Brown[8] reported three cases of "chronic pancreatitis" in which the diagnoses were confirmed by operation. The urines contained 500, 750 and 1,500 units, respectively (1,500 to 12,000 is considered the normal by his method). Corbett,[11] using Wohlgemuth's modified method, found 100 diastase units in the urine of a case with the clinical diagnosis of chronic pancreatitis. He reports 200 to 400 units in the urine of a case of miliary tuberculosis, showing a fibroid pancreas at necropsy, but without evidences of pancreatic disease during life. One hundred to 500 units were present in the urines of a patient with jaundice, glycosuria, diarrhea, epigastric pain, loss of weight, and stools containing 96 per cent.

15. Hirschberg: Zur Funktionsprüfung des Pankreas, Deutsch. med. Wchnschr., 1910, **36,** 1992.

16. Ehrmann and Kruspe: Untersuchungen über Pankreatitis chronica und Icterus chronicus, Ztschr. f. klin. Med., 1913, **78,** 122.

unsplit fat. This patient showed no evidences of either gallstones or carcinoma of the pancreas at operation. Spriggs and Leigh[17] found 20 diastase units in the urine in a case of chronic pancreatic insufficiency with fatty stools in which careful metabolic studies were made.

To summarize: eight cases with confirmed and three with the unconfirmed diagnosis of chronic, nonmalignant disease of the pancreas have been collected. The diastatic activity of the urine in eight of these cases varies from none to the usually accepted normal. Corbett's three cases were above the so-called normal limit. Corbett's case, with 500 units, is the only one in which the figure for diastatic activity is greater than we or others have found in cases without pancreatic disease. The maximum figure reported for cases without pancreatic disease is 400 units by the method used by Corbett.

The figures given for the diastatic activity in the urines in cases of cyst of the pancreas do not exceed those found by us or reported in the literature for patients without disease of the pancreas. Neumann[18] reported 20,000 diastase units in the urine in a case of pancreatic cyst. This figure becomes 200 units when calculated according to the terms of Wohlgemuth's modified method. Stocks,[12] Humphrey[14] and Brown[8] found moderate diastatic activity in the urines in five cases of cyst of the pancreas. All of these patients came to operation.

THE URINE IN TRAUMA TO THE PANCREAS

Noguchi[2] studied the diastatic activity of the blood and urine in three patients in whom injury occurred to the pancreas as a result of operative procedures. In the first case the pancreas was manipulated during the removal of a tumor from that region. Sixty hours after the operation the urine contained 64 diastase units by the modified and 160 units by the original method of Wohlgemuth. The pancreas of the second patient was superficially injured over an area about *"Fünfundzwanzigpfennig"* in size. At the end of twenty-four hours the urine showed 256 units by the modified and 320 units by the original method. A "rather large part" of the pancreas was resected in the third case. The urine twenty-four hours after the operation contained 512 diastase units by the modified and 1,280 units by the original method. The results in these three cases, together with comparable findings in experimental pancreatic lesions in dogs, to be discussed later, led Noguchi to believe that the amount of diastatic power in the urine depended on the extent of injury to the pancreas.

17. Spriggs and Leigh: A Case of Pancreatic Insufficiency, Quart. Jour. Med., 1913, **9**, 11.

18. Neumann: Das diastatische Ferment des Urins, Deutsch. Arch. f. klin. Med., 1913, **111**, 164.

THE URINE IN ACUTE PANCREATITIS

Hirschberg,[15] using Wohlgemuth's original method, reported 400 diastase units in the urine in a case of acute pancreatic necrosis twelve hours after operation. Two hundred to 250 units were present during the succeeding two weeks and 100 to 150 units during convalescence. In a second case the urine contained 1,600 units "immediately" after operation and on the second and third days thereafter 55 and 130 units. Marino[18] in one case found 200 diastase units in the urine. Lindemann[19] made numerous estimations of the diastatic activity in the urines of three operated cases. Two were acute pancreatic necrosis and one a "retroperitoneal abscess, which reached up to the pancreas." He assumed that pressure of the abscess occluded the pancreatic duct. The diastase in the urines in these cases ranged from 500 to 1,660 units. As the patients convalesced the diastatic activity decreased, but varied between 100 and 250 units. Corbett,[11] using Wohlgemuth's method, reported 66 diastase units in a case of acute pancreatic necrosis in which the diagnosis was confirmed by operation. Of seven suspected cases, the urines contained 100 units in two and 50 units in five.

Using the modified method, we have estimated the diastatic power of the urines in four cases of acute pancreatic disease. The diagnoses were verified by operation. Brief reports of these cases follow:

REPORT OF CASES

CASE 1.—*Acute pancreatitis.* This case occurred in the practice of Dr. F. J. Cotton. The symptoms were suggestive of acute cholecystitis. At the operation the peritoneum was studded with small points of fat necrosis. The lesser peritoneal cavity contained a mass of detritus and some pus. The pancreas, as such, was not distinguishable. After the operation large masses of necrotic tissue were discharged through the abdominal wound. Microscopically, this necrotic tissue was found to be largely fat. After a long period of convalescence the wound healed. Two urines in this case, collected one and two weeks after the operation, contained 40 and 100 diastase units.

The following three cases occurred on the surgical service of the Massachusetts General Hospital:

CASE 2.—*Abscess of the pancreas and acute pancreatitis.* J. A. R., aged 40, No. 193241. For two years the patient had recurrent attacks, twelve in all, characterized by abdominal distention, pain and vomiting, coming on a half to an hour after a heavy meal. The duration of these attacks varied from a few hours to a week. The last attack began five days before entrance to the hospital. Physical examination showed the abdomen to be distended. There was tenderness in the epigastrium. In the left hyochondrium was a large, rounded, smooth, slightly tender, resilient mass. The urine contained sugar. At the operation an abscess of the pancreas containing a quart of pus was opened and drained. Eight diastase units were present in the urine the sixth day after operation.

CASE 3.—*Acute hemorrhagic pancreatitis.* J. M. M., aged 40, No. 190582. The onset was sudden. There was constant severe epigastric pain and vomiting.

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19. Lindemann: Zur diagnostischen Bedeutung des Diastasegehaltes in Urin und Stuhl, Ztschr. f. klin. Med., 1912, **75**, 58.

Physical examination elicited marked muscular spasm and tenderness in the right upper quadrant and epigastrium. The temperature on admission was 98.6 F.; leukocytes 27,000 per cmm. The urine was normal. At the operation the head of the pancreas was found to be hard, swollen, edematous and red. Throughout the illness the temperature varied from 98.6 to 100 F. The drainage wound closed after two weeks. The urine one week after the operation contained 20 diastase units.

CASE 4.—*Acute hemorrhagic pancreatitis.* J. F. Mc., aged 53, No. 190582. The onset was sudden. At first there were moderately severe colicky pains in the epigastrium. Within twenty-four hours the pains became very severe and were accompanied by vomiting. Physical examination showed tenderness and muscular spasm in the upper half of the abdomen. This was most marked in the epigastrium. The admission temperature was 100 F.; leukocytes 27,000 per cmm. At the operation, August 7, the pancreas was found to be a bloody, edematous mass. The patient lived one month. During this time the drainage wound constantly discharged. The temperature during the illness varied from 99 to 103 F. The urine examinations are given in Table 3.

TABLE 3.—DIASTATIC ACTIVITY OF THE URINE IN HEMORRHAGIC PANCREATITIS *

Date	Amount Urine, C.c.	Diastase Units in 1 C.c. Urine	Fehling's Solution	Glucose in Urine, Gm.	Diastase Units in 1 Gm. Feces
8/ 9	Morning specimen	40	Reduced		
8/ 9	12-hour specimen	26	Reduced		
8/11	1,300	6	Reduced	37.8	
8/12	500	8	Reduced	10.4	
8/16	Single specimen	200	Not reduced	....	10
8/18	Single specimen	20	Not reduced		

* Operation August 7.

Eight cases of suspected acute pancreatitis and six with the diagnosis confirmed by operation have been collected. To the latter we add four cases. Two cases of direct operative injury to the pancreas are reported by Noguchi.[8] Of the ten cases of acute pancreatitis with confirmed diagnoses, the diastatic activity in the urines in seven was on certain examinations higher than the usually accepted normal. In the urines of two patients the figures for diastase units were higher than those reported either in our own series or in the literature for patients without evidences of pancreatic disease. In the latter case 500 units are the maximum reported, while in Lindemann's[10] two cases of acute pancreatitis, 1,660 units were present. One of our patients showed values of 100 units two weeks after operation and another 200 units ten days after. These figures are not higher than found in 7 per cent. of our series without pancreatic disease, and in 4 per cent. collected from the literature. Lindemann assumes that the high diastatic activity in the urine of his patient with "retroperitoneal abscess" was due to the pressure of the abscess on the duct of the pancreas.

On the other hand, Noguchi's[3] case, in which the pancreas was directly manipulated but not noticeably injured, showed no increase in the diastatic power of the urine. In his two cases with direct injury to the pancreas the figures given for the diastase units by the modified method are high. Unusually high figures were obtained in but one of these cases. Thus of the thirteen cases of acute pancreatic affection five give figures for the diastase which are higher than found in cases without pancreatic disease. In these cases the figures given are 512 units by Wohlgemuth's modified method in one case, and from 1,600 to 1,660 units by the original method in four cases.

SUMMARY OF THE CLINICAL STUDIES ON THE URINE

The diastatic activity of the urine has been estimated by Wohlgemuth's modified method in 346 patients without disease of the pancreas; 95.6 per cent. of these cases showed from zero to 80 diastase units in the urines. In 450 cases without pancreatic disease, the diastatic activity of the urine was estimated by the original method; 95.3 per cent. of these cases showed from zero to 200 units in the urine.

The diastatic activity of the urine was determined by one of Wohlgemuth's methods in sixty-one cases in which the pancreas had been diseased, injured, or manipulated. This number includes our cases and those collected from the literature.

In forty-two of these the diagnoses were confirmed by operation or necropsy. In five the figures given for diastatic activity in the urine are greater than those reported for patients without pancreatic disease. In Noguchi's[3] case of resection of part of the pancreas the patient showed 512 units, while 400 units[1] is the highest reported for that method in cases without pancreatic disease. Lindemann's[19] case of "retroperitoneal abscess reaching up to the pancreas" showed 1,660 diastase units in the urine. Hirschberg's[15] one case and Lindeman's[19] two cases of acute pancreatitis gave 1,600 and 1,660 diastase units in the urine, while the maximum figure obtained in cases with normal pancreas by the method used by these investigators is 500 units.[20]

The diagnosis of pancreatic disease was unconfirmed by operation or necropsy in nineteen cases. The figures given for the diastatic activity of the urines do not exceed those found in cases without pancreatic disease, except in two cases reported by Wohlgemuth[21] with "total, or at least partial, occlusion of the pancreatic duct," in which the urine contained 1,250 and 625 units.

In seventeen cases, or 40.5 per cent. of the forty-two cases with

20. Wynhausen: Zur Funktionsprüfung des Pankreas, Berl. klin. Wchnschr., 1910, **47,** 478; 1909, **46,** 1406. Geyelin: A Clinical Study of Amylase in the Urine, THE ARCHIVES INT. MED., 1914, **13,** 96.

21. Wohlgemuth: Ueber eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments, Biochem. Ztschr., 1908, **9,** 1.

verified diagnoses, a high diastatic activity was present in the urines. From 100 to 512 diastase units were present in the urines of seven of the cases studied by Wohlgemuth's modified method. By his original method the urines in seven cases contained from 250 to 1,660 diastase units and in three cases contained 200 units. As a high diastatic activity occurred in the urines in from 4 to 7 per cent. of cases without evidences of pancreatic disease, this finding is of limited value as an aid in the diagnosis of disease of the pancreas.

THE URINE IN EXPERIMENTAL PANCREATIC LESIONS

Wohlgemuth[22] describes the effect on the diastase in the blood and urine of tying off the principal pancreatic ducts in three animals. An increase in the diastatic power of the urine occurred within twenty-four hours and persisted several days. In the first animal, a rabbit, 40 diastase units were present in the urine before the operation of tying the ducts. During the six days following the operation 500, 780, 500, 780, 200 and 40 units were found. In the second animal, a dog, the urine possessed no diastatic activity before the operation. During the succeeding six days 80.5, 625, 312.5, 805, 10 and 5 units were present. In another dog a "small portion" of the tail of the pancreas was tied off. Before the operation the urine contained 10 diastase units and during the five days following the operation 50, 200, 125, 50 and 20 units were present.

In two dogs the ductus choledochus was tied and cut. Before the operation the urines contained no diastatic power. The figures for the diastase units the nine days following the operation are 0, 0, 0, 5, 80.5, 625, 156, 40 and 20 units for one dog, and 5, 5, 20, 10, 40, 156, and 20 units for the other dog.

These results at first sight seem to indicate that some factor is involved in producing an increase in the diastatic power of the urine other than that of injury to the pancreas. Wohlgemuth offers two possible explanations. One is that the pancreatic duct may have been closed through the formation of scar tissue. But it would not be possible for newly formed connective tissue to constrict the pancreatic ducts in such a short time. Further, he suggests that there may be some nervous relation between the ductus choledochus and the pancreatic ducts which results in a functional constriction of the pancreatic ducts when the bile duct is tied. This hypothesis is unsupported by any evidence. As an increase in the diastatic activity of the urine is known to result from acute pancreatitis, it is more probable that some inflammation of the pancreas secondary to infection about the common duct explains the rise noted.

22. Wohlgemuth: Beitrag zum Verhalten der Diastase im Urin, Biochem. Ztschr., 1909, **21**, 432.

Wohlgemuth and Noguchi, using the modified method, studied the increase in diastatic power in the urine in relation to the extent of trauma produced on the pancreas of two dogs. In the first animal the pancreas was cut across. The urine contained no diastatic power before the operation. Five hourly examinations following the operation showed 8, 16, 16, 32, and 64 units. In twenty-four hours 256 units, and in forty-eight hours 512 units, were present.

The second animal's pancreas was traumatized by making a small stab wound with a scalpel and lightly squeezing the neighboring pancreatic tissue with the fingers. Before the operation 2 diastase units were present in the urine. Six one-hour examinations after the operation showed 2, 2, 8, 16 and 16 units. Thirty-two units were present in a specimen collected at the end of twenty-four hours, and 32 at the end of forty-eight hours. The authors conclude that the time of appearance and magnitude of the increase in diastatic power of the urine varies directly with the extent of trauma in experimentally-produced lesions of the pancreas. But it is to be noted that Wohlgemuth[22] found variations in the diastatic activity in the urines of normal dogs of from none to 78 units, using his original method.

We have studied the diastatic activity in the urines of nine dogs. In six of the animals the pancreas was traumatized. In one a pancreatic graft was placed in the spleen. Two dogs were narcotized and no operative procedures carried out. The results obtained were as follows:

Dog 1.—Female; weight 12.5 kilos; ether anesthesia; operation August 16. All of the pancreas except the processsus uncinatus was removed. The dog rapidly declined in strength and dropped to 9.3 kilos in weight the fourth day. A severe glycosuria developed on the third day (Table 4). No diastatic activity was present in the duodenal contents obtained after death, August 22.

TABLE 4.—Dog 1. Operation August 16

Date	Amount of Urine in 24 Hours	Diastase Units in 1 C.c. Urine	Diastase Units in 24-Hour Urine	Diastase Units in 1-Gm. Stools
8/13	20	0		
8/14	40	0	.....	7
8/16	1,000	2	2,000	10
8/17	100	0.66	66	
8/18	250	40	10,000	
8/19	400	100	40,000	
8/20	330	8	1,200	
8/21	150	8	1,200	
8/22	600	0	.....	10

Dog 2.—Female, weight 4 kilos; ether anesthesia; operation August 15. Double ligatures were placed around the pancreas at the junction of the processus lienalis and corpus, and of the processes uncinatus and corpus.

TABLE 5.—Dog 2. Operation August 15

Date	Amount of Urine in 24 Hours	Diastase Units in 1 C.c. Urine	Diastase Units in 24-Hour Urine	Diastase Units in 1-Gm. Stools
8/11	450	2	900	10
8/14	....	1	.....	40
8/16	20	20	400	
8/17	150	200	30,000	
8/18	250	200	50,000	
8/19	200	40	8,000	10
8/21	300	0		
8/22	300	4	1,200	
8/23	300	4	1,200	

Dog 2 was subjected to a second operation under ether anesthesia, August 27. The pancreas was found to be firmer to touch than normally, but was macroscopically otherwise unaltered. A small piece of pancreas 3 cm. in length was excised and another portion was placed in a pocket made in the spleen.

TABLE 6.—Dog 2. Operation August 27

Date	Amount of Urine in 24 Hours	Diastase Units in 24-Hour Urine	Diastase Units in 1 C.c. Urine
8/28	15	800	50
8/29	82	164	2
8/30	70	46	0.66
8/31	400	800	2

Dog 3.—Female; weight 8.5 kilos; operated on under ether anesthesia, August 27. Three pieces of pancreas, freshly excised from another dog, were imbedded in the spleen. The pancreas of Dog 3 was not manipulated.

TABLE 7.—Dog 3. Operation August 27

Date	Amount of Urine in 24 Hours	Diastase Units in 24-Hour Urine	Diastase Units in 1 C.c. Urine
8/28	80	.....	0
8/29	10	1,000	100
8/30	550	2,200	4
8/31	550	2,200	4

That pieces of pancreatic tissue imbedded in the spleen rapidly undergo autolysis Pratt and Murphy[23] have found. The experiment on Dog 3 clearly shows that destruction of foreign pancreatic tissue may produce an increase in the diastatic activity of the urine.

In Dog 5 all of the pancreas but a portion 3.5 by 3 cm. of the processus uncinatus was excised. A pancreatic graft 3 by 5 cm. was placed in the spleen and an emulsion of pancreatic tissue also injected into the spleen. No rise in diastatic activity in the urine occurred in the five days succeeding the operation.

Two dogs from which all of the pancreas but the processus uncinatus was removed showed no diastatic power in the urines for the forty-eight hours succeeding the operations. From the second to the fifth day following a similar operation in another dog the diastase values were 10, 4, 2, and 1 units per c.c. of urine.

The effects of ether anesthesia on the diastatic activity of the urine were studied. Two dogs were narcotized with ether for two and a half hours. The urines for the following twenty-four hours contained 30 and 20 diastase units per c.c., and those on the succeeding day 1 and 4 units. As Wohlgemuth,[22] using his original method, found 7.8 to 78 diastase units in the urine of a normal dog, it is possible that the figures found in our two narcotized dogs represent nothing more than normal variations, but it is more probable that the administration of ether was the cause of the relatively high diastatic values.

In two of our animals (Tables 4 and 5) an increase in the diastatic activity of the urine occurred in from one to two days following injury to the pancreas. This increase persisted from one to four days.

Our findings are materially different from those of Wohlgemuth and Noguchi. They observed an increase in the diastatic activity in the urine in every animal examined in which the pancreas was injured, while none was present in the urine of two of our dogs. In all of their experiments the maximum increase occurred on the second day after the operation, while in our series Dog 1 had the greatest increase on the third day and Dog 2 on the first day after the second operation. The increase was less in our experiments than in those reported by these investigators. Only one of our dogs had 200 units of diastase in the urine. Wohlgemuth observed a rise to 805 in one of his experiments and 780 in another.

DIASTATIC ACTIVITY OF THE FECES

The diastase found in the feces is probably a product of the secretion of the pancreas. The exclusion of the pancreatic juice from the intestines is followed by a marked diminution of the diastatic activity

23. Pratt and Murphy: Pancreatic Transplantations in the Spleen, Jour. Exper. Med., 1913, **17**, 252.

of the feces. This has been demonstrated by the experimental work of Wohlgemuth,[1] Wynhausen,[2] and ourselves. That ptyalin of saliva is not a source of the diastase in the feces has been shown by Wohlgemuth.[1] This investigator tied the pancreatic ducts in dogs. After the operation no diastatic activity was demonstrable in the feces. He then fed the dogs human saliva, but the stools still remained free from diastatic activity. Fuhrmann[24] and Lifschütz[25] have shown that the bacteria of the feces can produce but a very little diastase.

It has been considered probable that the estimation of the diastatic activity of the feces would give information regarding the functional condition of the pancreas. Destructive lesions of the pancreas or occlusion of the duct of Wirsung would be expected to be accompanied by a decrease in the amount of pancreatic juice entering the intestines. This has led investigators to attempt to establish a minimum normal for the amount of diastatic activity possessed by the feces.

Wynhausen[2] reported a minimum of 500 diastase units and a maximum of 20,000 units from the study of 170 stools. He considers 500 units the minimum normal limit. Hirschberg[15] states that "a number of patients in whom the pancreas was normal" gave from 200 to 500 units of diastase in the stools. Lindemann[19] found a minimum of 100 units and a maximum of 2,282 units in the stools of twenty-four patients without evidences of pancreatic disease. Bálint and Molnár[26] state that "investigations on normal persons show great variations" in the diastatic activity of the stools, but give no figures. They report three cases of Basedow's disease in which the diastatic activity of the stools ranged from 300 units to 100,000 units. Hirayama[27] reported from zero to 150 diastase units in the stools in twenty-nine patients without pancreatic disease. Friedmann[28] studied the diastatic activity in the feces of seventeen patients without disease of the pancreas. The figures given are 25 to 50 diastase units in four cases, 250 to 600 units in four, 5,000 units in seven, and 50,000 units in one. Arnold[29] found from 125 to 780 units in the feces in six cases of intestinal fermentative dyspepsia without evidences of diseases of the pancreas. All of

24. Fuhrmann: Vorlesungen über Bakterienenzyme, Jena, 1907, 84.

25. Lifschütz: Zur Frage der funktionellen Diagnostik der Pankreaserkrankungen, Arch. f. Verdauungskr., 1913, **19,** 562.

26. Bálint and Molnár: Zur Pathogenese der Diarrhöen bei Morbus Basedowii nebst Bermerkungen über diagnostische Bewertung des Fermentsgehalts der Faeces, Berl. klin. Wchnschr., 1910, **47,** 1620.

27. Hirayama: Ueber den Gehalt der Faeces an tryptischen und diastatischen Ferment bein normaler Verdauung im Fieber und im diarröischen Stuhl, Ztschr. f. exper. Path. u. Therap., 1911, **8,** 624.

28. Friedmann: Quantitative Estimation of Trypsin and Amylase in the Feces and Duodenal Contents, Med. Rec., New York, 1912, **81,** 355.

29. Arnold: Ueber den Diastasegehalt der Fäces bei Gärungsdyspepsie, Zentralbl. f. inn. Med., 1913, **1,** 34, 1.

these investigators used Wohlgemuth's original method. Rotky,[30] writing in 1913, considers the establishment of a normal as impossible from the results given by different authors.

Altogether, figures for the diastatic activity of the stools of 350 patients have been collected from the papers of Wynhausen,[2] Hirschberg,[15] Lindemann,[19] Bálint and Molnár,[26] Hirayama,[27] Friedmann,[28] and Rotky.[30] No diastatic activity was present in the stools of nineteen of these patients.[27] In the stools of seven patients there were from 25 to 50 diastase units. In the stools in 302 cases from 200 to 20,000 diastase units were present. Friedmann[28] reported 50,000 diastase units in the stools in one case, and Bálint and Molnár[26] 100,000 in another. The stools in forty-one, or 11.7 per cent. of the 350 cases, contained from none to 200 diastase units. Brown[8] modified Wohlgemuth's technic and studied the diastatic activity in the feces of fifteen healthy men and women. By Brown's method the number of diastase units contained in the entire amount of feces collected is computed. Variations of from 60,000 to 240,000 diastase units were found. He considers 60,000 units as representing the minimum normal limit. Brown's method consists of a special meal of 750 c.c. of milk given at 7 a. m., and followed by ½ ounce of magnesium sulphate at 7:30 a. m., and again at 8 a. m. At 8:30 a. m. a glass of water containing ¼ teaspoonful of sodium bicarbonate is taken. The stools are collected up to 2 p. m., and if less than 400 gm. are obtained, an enema of a pint of water is given. The preceding evening the patient receives a high enema and is allowed only a very light supper.

The feces are collected over a period of seven hours. Certainly stools passed within the first few hours cannot result from the milk ingested. We found large amounts of vegetable fiber in the stools of two patients on whom Brown's method had been carried out. This came from vegetables eaten the day previous to the test. It is questionable how often the milk reaches the rectum in the seven hour period. Roentgenographers consider that it takes the bismuth meal used in gastro-intestinal studies from seven to nine hours to pass into the cecum. Hertz[31] has shown that the magnesium sulphate does not affect the motility of the small intestines, but acts only on the colon. Hence, by Brown's technic, the magnesium sulphate will have spent its effect on the colon before the milk meal has reached the cecum. To test this point the technic of the method was carried out on three normal adults. Forty grams of bismuth were added to the milk taken by two of these. Roentgenograms at the end of seven hours showed the bismuth to have

30. Rotky: Ueber den Diastasegehalt der Faeces, München. med. Wchnschr., 1913, **60**, 2158.

31. Hertz, A. F.: Constipation and Allied Intestinal Disorders, London, 1909, pp. 9 and 279.

reached the ileocecal valve in one, and to have just entered the transverse colon in the other case. Enemata at the end of eight hours in both these cases produced stools without bismuth. Seventy-five grams of bismuth were added to the milk given to the third normal person. At the end of seven hours a roentgenogram showed that the bismuth had reached the sigmoid colon, but the major part of the bismuth was in the terminal ileum and cecum. An enema at the end of seven and a half hours yielded a stool which contained no bismuth. Brown's method was also carried out without the use of bismuth on three adults who had no gastro-intestinal disturbance. One gram of charcoal was added to the milk. At the end of seven hours the stools of only one patient contained charcoal. But if a stool resulting from the milk is obtained it will be mixed with (1) the contents of the colon not removed by the high enema of the preceding evening; (2) the unabsorbed residue of the light supper; (3) and often the remains of food in the stomach or small intestines eaten prior to this meal.

Using Wohlgemuth's modified method, Stocks[12] estimated the diastatic activity in the stools of a normal person and in two cases of diabetes mellitus. A stool from the normal man contained 38 diastase units. Three stools were collected on different days from one of the diabetics. The stools of this patient contained 21.3, 750, and 434 units. The stools of the other contained 5 units. Neumann[13] found no diastatic activity in the stools in a case of catarrhal jaundice.

We have estimated the diastatic activity of the stools of sixty-eight patients without evidences of disease of the pancreas. These cases embrace fifty-two nonfebrile and two febrile diseases, that is, typhoid fever and pulmonary tuberculosis. The diet of fifty-seven of these patients was the "general hospital diet." The stools were the result of either magnesium sulphate or castor oil. In four cases the stools were diarrheal. The results are given in Table 8.

TABLE 8.—THE STOOLS OF PERSONS WITH NORMAL PANCREAS

Diastase Units in 1 Gm. Feces	Number of Examinations	Number of Cases
0	10	7
7 to 10	13	12
20 to 50	20	13
200 to 800	17	17
1,000 to 5,000	13	13

The stools in seven cases, or 13.2 per cent. (sprue, hepatic cyst, intestinal infantilism, chronic nephritis, typhoid fever), contained no diastatic power. In thirty-two of these cases, or 51.6 per cent., the

stools contained less than 50 diastase units. Stools collected on different days were examined for the diastatic activity in thirteen cases. It was found that variations exist in the diastatic power of different stools from the same case. That this variation is often great is shown in Table 9.

THE FECES IN MALIGNANT DISEASE OF THE PANCREAS

Using Wohlgemuth's original method Wynhausen[2] reported 10 to 30 diastase units in the stools in two cases of carcinoma of the pancreas. The diagnoses in both cases were confirmed by necropsy. Wynhausen[2] found 12.5, 20 and 30 diastase units in the "different" specimens of feces from a patient with a tumor in the head of the pancreas. At operation this tumor was found to occlude the duct of Wirsung. The character of the tumor is not stated. Using his own technic, Brown[8] reported 1,200 diastase units in the stools in four cases of carcinoma of the pancreas. The diagnosis in two of these cases was verified by operation.

TABLE 9.—VARIATIONS IN DIASTATIC ACTIVITY IN STOOLS FROM THE SAME PATIENT

Diastase Units in 1 Gm. Feces	Number of Examinations	Number of Cases	Diagnosis
0 to 20	4	2	Hepatic cyst; typhoid fever
0 to 70	6	2	Sprue; intestinal infantilism
7 to 400	9	4	Neurasthenia; chronic nephritis; chronic appendicitis
40 to 400	2	1	Sciatica
7 to 2,000	2	1	Suppurative sinusitis
400 to 2,500	4	2	Secondary syphilis; carcinoma of prostate
1,000 to 5,000	2	1	Neurasthenia

We have estimated the diastatic activity of the stools in the three cases of carcinoma of the pancreas previously discussed in the section on the urine in this disease. These patients came to operation or necropsy. They were all jaundiced. The feces contained large quantities of fat and a small amount of undigested muscle of fiber and starch granules. No diastatic activity was present in the feces of one patient. In a second case the stool contained 40 diastase units. Seven and no diastase units were present in two stools, collected on different days, from the third patient. At the necropsy in this case the duct of Wirsung was found to be completely occluded.

In our three cases of carcinoma of the pancreas and in the two reported by Wynhausen the diastatic activity in the feces is low. Nevertheless, the figures are not lower than those obtained by

Hirayama,[27] Friedmann,[28] and ourselves in patients without disease of the pancreas. Because of the method used by Brown, his figures do not admit of comparison with those obtained by other writers, but the values given are low.

THE FECES IN CHRONIC PANCREATITIS

Using Wohlgemuth's original method, Hirschberg[15] reported no diastase and 150 units in two stools in a case of chronic interstitial pancreatitis which came to necropsy. The case of Ehrmann and Kruspe,[10] discussed earlier in this paper, contained "repeatedly small amounts of ferment, but later frequently normal or increased content of the diastase." After a return to "normal" diastatic activity the azotorrhea persisted. No figures for diastatic activity are given. Brown[8] estimated the diastase activity of the stools in six cases of "chronic pancreatitis." The diagnoses were all verified by operation; "a small hard, diseased organ being found in each case." The diastase units in each case were 8,000, 7,500, 12,000, 8,000, 7,500 and 3,300 units. These figures cannot be compared with those of other writers, because of the difference in the method used. The values are low, however, since Brown considers 60,000 units the minimum normal limit. The diastatic activity in the feces of all these reported cases of chronic pancreatitis is low. But it is not lower than has been found in cases without pancreatic disease. The feces of forty-one, or 11.7 per cent. of these patients, contained less than 200 diastase units.

THE FECES IN ACUTE PANCREATITIS

Using Wohlgemuth's original method, Hirschberg[15] estimated the diastatic activity in the stools in two cases of acute pancreatic necrosis. During the third week after operation the feces in one case contained 85 units and "during convalescence" 185 units. The stools in the second case contained from none to 46.5 units during the first week following the operation. On the seventh day a stool resulting from castor oil contained 1,000 units. When the stools "became of normal consistency, from 200 to 250 diastase units were found." Wynhausen[2] reported thirty units in the stools in two cases of acute hemorrhagic pancreatitis. It is not stated that the diagnoses in these two cases were confirmed by necropsy or operation. Lindemann[19] made numerous estimations of the diastatic activity in the feces in two cases of acute pancreatic necrosis and in a case of a "retroperitoneal abscess reaching up to the pancreas." In the first case of acute necrosis the stools contained from 70 to 28.7 diastase units in the period between the second and nineteenth day following the operation. From the fifth to the seventh week the figures range from 532 to 287.5 units. Nine days after operation the stools in the second case contained 100 diastase

units. On the twelfth day following the operation a slough of pancreatic tissue was expelled through the operation wound. During the next week from 1,000 to 660 diastase units were present in the feces. The stools in the case of a "retroperitoneal abscess" contained five days prior to operation, 99.5 diastase units; three days prior, 237 units, and the day before, 250 units. Lindemann assumed that the pancreatic duct was occluded by pressure of the abscess. At operation an ileac fecal fistula was made. The fistulous discharge contained from 2,286 to 5,375 units. In one case Lindemann[19] made the diagnosis of pancreolithiasis. The diagnosis was not confirmed by operation or necropsy. The stools in this case contained from 8 to 17 units during the first five weeks of the illness; 625 to 1,000 units were present in the stools during the two succeeding two weeks. Crohn[32] found no diastatic activity in the duodenal contents or stool in a case of acute pancreatitis.

We have estimated the diastatic activity of the stools in Cases 1 and 4 of acute pancreatitis. These cases were discussed in the section on the urine in acute pancreatitis. The stools were liquid in character. Otherwise they were macroscopically and microscopically normal. No diastatic activity was present in the stools in the case of acute pancreatic necrosis two and four weeks after operation; 10 diastase units were found in the stool in the case of acute hemorrhagic pancreatitis nine days after operation.

In nine cases of acute pancreatitis the diastatic activity of the feces has been estimated. Low values were found in all. In a case of Lindemann's[19] and one of Hirschberg's[15] the diastatic activity increased during convalescence. The same was true in Lindemann's case of pancreolithiasis.

SUMMARY OF THE CLINICAL STUDIES ON THE FECES

The diastatic activity of the feces is reported in twenty-three cases of pancreatic disease in which the diagnoses were verified by operation or necropsy. In three additional cases the diagnoses were not confirmed. These twenty-six cases include those collected from the literature and the ones reported by ourselves. The values given for diastatic activity cannot be compared on a numerical basis because of the differences in the methods used. But they are all low. The diastatic activity although low, does not differ from that found in the stools in patients without disease of the pancreas reported by us or reported in the literature. The stools of forty-one persons, or 11.7 per cent. of those without disease of the pancreas, collected from the literature, contained from zero to 200 diastase units. Thirty-two, or 51.6 per

32. Crohn: The Functional Activity of the Pancreas, Am. Jour. Med. Sc., 1913, **145**, 393.

cent., of our cases showed from none to 50 diastase units in the feces. The diastatic activity remained low in but four of these cases in which more than one stool was examined. The finding of low values in such a large percentage of persons with normal pancreas detracts greatly from the value of the estimation of the diastatic activity of the feces as an aid in the diagnosis of diseases of the pancreas. No one has made a large number of estimations in cases without pancreatic disease in which there was a greatly diminished or absent diastatic activity of the feces. It is quite possible that at times high values for the diastatic power would be found in such cases. This is made probable by the great fluctuation in the diastatic activity found in the stools in thirteen of our cases (Table 9). Lindemann[10] found similar variations in the stools of seven persons without pancreatic disease. A large number of estimations of diastatic activity have been made in the stools in five cases of pancreatic disease. In two cases of acute pancreatitis reported by Lindemann, persistently low values were found. In three other cases variable figures are reported. These three cases are discussed in the section on the factors that influence diastatic activity.

THE FECES IN EXPERIMENTAL PANCREATIC LESIONS

Wynhausen cut and tied the "pancreatic ducts" in a dog and partially separated the pancreas from the duodenal wall. After the operation there were "large amounts of diastase in the feces (500 and more units)." At a second operation three weeks later "part" of the pancreas was completely normal and "part" showed a marked "pancreatitis." A patent duct communicating with the duodenum was found. This was tied and cut. A considerable part of the pancreas was extirpated and the remaining portion was cauterized with the Paquelin cautery. The dog developed glycosuria and lost weight. Two days after this second operation the feces contained 500 diastase units, one week later 10 units, and on subsequent examinations, 12.5 and 5 units.

Wohlgemuth makes the statement that no diastatic activity is present in the feces of dogs in which the pancreatic ducts are occluded, while the feces of normal dogs contain a high diastatic activity. He gives no figures or protocols and the number of dogs examined is not stated.

We have studied the diastatic activity in the feces of three normal dogs and, also, in two of these animals after excluding pancreatic secretion from the duodenum. One of the dogs (Table 4) showed 7 diastase units in the stools before exclusion of pancreatic juice from the intestines, while six days after the operation 10 units were present. The dog passed stools in the intervening period, but they were lost in the urine. On the same day that the last stool examination was made

the dog was killed. No diastatic activity was present in the duodenal contents collected at the necropsy.

In Dog 2 (Table 5), 10 and 40 diastase units were present in the stools prior to the operation in which ligatures were placed around a portion of the pancreas. Pancreatic juice was not excluded from the intestines. On the fifth day following the operation the stools contained 10 diastase units.

In another of our dogs 200 diastase units were present in the stools nine days before the operation. The pancreas was completely separated from the duodenal wall and all of the gland except the processus uncinatus extirpated. No diastatic activity was found in the feces of this dog on the sixth day following the operation. The contents of the duodenum and jejunum removed at the necropsy of this animal showed no diastatic activity.

From these findings the conclusion is justified that exclusion of pancreatic secretion from the intestines in dogs is followed by a marked diminution, or by an absence, of the diastatic activity in the stools.

FACTORS INFLUENCING THE DIASTATIC ACTIVITY OF THE FECES

That a lack of pancreatic secretion is not the only cause of a low diastatic activity in the feces is suggested by the following observations:

1. The diastatic activity may be low or absent when the pancreas is normal. The stools in 11.7 per cent. of the patients with normal pancreas collected from the literature contained less than 200 diastase units; nineteen showed no diastase and seven less than 50 units. In nine of our sixty-eight cases with normal pancreas, the stools contained no diastase, and in fifteen the number of units was 10 or less.

2. Great variations occur in different stools from the same patient without any change in diet or in the patient's apparent condition. This is shown in Table 9.

3. The diastatic activity in the contents of the small intestine may be high and yet that of the stools low. In Lindemann's case of "retroperitoneal abscess" the intestinal contents discharged from the fistula contained from 2,286 to 5,375 diastase units, while during the week prior to operation from 99.5 to 250 units were present in the feces. Werzberg[33] examined the diastatic activity of the intestinal contents in a case of carcinoma of the pylorus. At necropsy the pancreas was found to be normal. The contents of the duodenum and of the small intestines contained 1,533 diastase units, while the contents of the colon showed but 100 units.

33. Werzberg: Zur Diagnostik der Pankreaserkrankungen, Arch. f. Verdauungskr., 1911, **17**, 533.

Variations in the absorption of pancreatic juice from the intestines would influence the quantity of diastase in the feces. Boldyreff[34] has shown that pancreatic secretion is normally absorbed from the intestines in dogs. Variations in the secretion of pancreatic diastase could produce corresponding differences in the diastase content of the feces. Crohn[32] states that the diastatic activity of the feces roughly corresponds to that of the duodenal contents. Both Crohn[32] and Holsti[35] found that the diastatic activity of different specimens of pancreatic juice varied widely. Crohn obtained pancreatic secretion by means of the duodenal tube, while Holsti made observations on a young man with a traumatic fistula. We have studied the effect of bacteria on the diastatic activity of eight stools from different persons. Portions of each stool were incubated at 38 C., refrigerated, and kept at room temperature for three days. Estimations of the diastatic activity were made at the beginning of the experiment and at the end of twenty-four, forty-eight, and seventy-two hours. The different portions of the same stools showed equal degrees of diastatic activity regardless of whether they were incubated, refrigerated, or kept at room temperature.

Strasburger[36] investigated the effect of different foods on the diastatic activity in the stools. His experiments were carried out on three persons without pancreatic disease. He concludes that the kind of food eaten has no effect on the diastatic power of the feces. Corbett[11] states that "the ordinary variations in hospital food from milk to full diet does not appear to have much influence on the output of diastase." Hawk[37] found that water drinking increased the diastatic power of the stools.

Strasburger[36] reported a large diastatic activity in the diarrheal stools from five patients. A small amount was found in the stools in two cases of constipation. From these findings he concludes that diarrhea causes an increase and constipation a decrease in the diastatic activity of the feces. We found in the stools of seven constipated persons with normal pancreas, no diastatic activity in five, 25 units in one and 3 units in one. In the case with 3 units two liquid stools resulting from magnesium sulphate each contained 3 units. Hirayama[27] reported from zero to 50 units in the stools of nine patients with diarrhea. We found from zero to 12 units in the diarrheal stools of seven patients

34. Boldyreff, W.: Einige neue Seiten der Tätigkeit des Pankreas, Ergebn. d. Physiol., 1911, **11**, 121.

35. Holsti: Beiträge zur Kenntnis der Pankreasserkretion beim Menschen, Deutsch. Arch. f. klin. Med., 1913, **3**, 48.

36. Strasburger: Weitere Untersuchungen über Faecesgaehrung nebst allgemeinen Bemerkungen über das diastatische Ferment im menschlichen Stuhle, Deutsch. Arch. f. klin. Med., 1900, **67**, 238.

37. Hawk, P. B.: The Activity of the Pancreatic Function Under the Influence of Copious and Moderate Water Drinking with Meals, THE ARCHIVES INT. MED., 1911, **8**, 382 and 552.

and 400 units in another. These findings strongly suggest that the absence or diminution of diastase in the feces is often the result of absorption of the pancreatic secretion. In constipation, as is well known, absorption of the digested food is unusually complete, and this explains the absence of diastase. Bacterial action, as we have shown, does not destroy diastase. In some cases of diarrhea the absorption from the liquid intestinal contents may be good, as metabolism studies may show a normal absorption of nitrogen, carbohydrates, and fat even when the peristalsis is very active. Hence, the absence of diastase found in diarrhea is probably due to its absorption and not to its destruction.

Alkalies, neutral salts and acids influence the action of ferments.[38] The effect of these substances on the diastatic activity of the feces has been studied. Hawk[37] states that the accelerating effect of acids is without importance in clinical studies. Rotky[30] studied the effect of different concentrations of sodium chlorid on the diastatic activity of the dried feces. Different concentrations of salt mixed with the same stool produce variations ranging from zero to 427 units. Ury[39] studied the diastatic activity in the dried stools of one normal person. He claimed that senna increased, and magnesium sulphate decreased, the diastatic activity of the feces. But many of the highest values found by us were present in stools resulting from the administration of magnesium sulphate.

The chief cause of a diminished diastatic activity of the feces, and the only one that is clearly understood, is an absence of pancreatic juice or a diminished amount in the intestine. This would occur when the pancreatic ducts are occluded or the pancreatic tissue extensively diseased. If the duct of Wirsung alone were closed, pancreatic secretion would enter the duodenum through the accessory ducts. Nevertheless, the reported cases of obstruction of the duct of Wirsung have been accompanied by a low diastatic activity in the feces.

A low or absent diastatic activity of the feces is not conclusive evidence of a diminution or a lack of pancreatic secretion entering the intestines, as it is found in many cases without pancreatic disease. Lindemann[19] considers that for diastase determinations to be of value in diagnosing disease of the pancreas there must be a high diastase content in the urine and a low content in the feces. This occurred in three of our cases with normal pancreas.

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38. Oppenheimer, C.: Die Fermente und ihre Wirkungen, Leipzig, 1909, Ed. 3, p. 102.

39. Ury: Ueber den quantitativen Nachweis von Fermenten in den Faeces, Biochem. Ztschr., 1909-1910, **23**, 143.

CONCLUSIONS

1. The estimation of the diastatic activity of the urine is of very slight value in the diagnosis of pancreatic disease. In rare instances, however, a large amount of diastase is present—more than 500 units per cubic centimeter. This finding strongly suggests disease of the pancreas.

2. The diastatic activity in the urines of persons with normal pancreas varies widely.

3. Injury to the pancreas of dogs may be followed by a marked increase in the diastatic activity of the urine, but this does not always occur.

4. Ether anesthesia may increase the diastase in the urine of dogs.

5. The estimation of diastatic activity in the feces is of slight value as an aid to the diagnosis of disease of the pancreas.

6. Obstruction of the pancreatic ducts in man results in a low diastatic activity in the feces.

7. The diastatic activity in the feces of persons without pancreatic disease varies widely.

8. The absence of diastatic activity in constipation and in other conditions, in which the pancreas is normal, is probably due to complete absorption of the diastase by the intestines.

9. Exclusion of pancreatic secretion from the intestines in dogs is followed by a marked diminution or absence of diastatic activity in the stools.

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THE SUPRARENAL SYSTEM AND CARBOHYDRATE METABOLISM *

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Prior to the experiments of Blum[1] in 1901, when he demonstrated that injections of a watery extract of the adrenal glands constantly give rise to glycosuria in dogs, very little attention had been given to the action of the suprarenal secretion on metabolism. Claude Bernard's[2] conception of an internal secretion enunciated in 1855, and the publication, in the same year, of Thomas Addison's book containing his observations on the association of a definite clinical syndrome with pathologic changes in the adrenal glands, prepared the way for Brown-Séquard's[3] well known experiments. His conclusions, though in part erroneous, were epoch-making. Because of the stimulus it supplied to investigations in the new field of internal secretion, the value of this work was tremendous. But so far as the adrenals were concerned, these early researches had been confined chiefly to studies of their effect on blood pressure, local changes in the tissues and blood vessels at the site of injection, and to general symptoms following extirpation experiments.

Abundant experimental data, and much speculation as to the rôle of the adrenal secretion in carbohydrate metabolism has accumulated in the fifteen years since Blum's observation. This work has, for the most part, represented efforts to discover the mechanism by which the suprarenal system, and more particularly the adrenal glands themselves, intervene in sugar metabolism, with the idea constantly present that they may be implicated in the perversions of metabolism in diabetes mellitus.

In addition, however, to the glycosuric effects of epinephrin injections, there are other observations which indicate that the adrenals affect the metabolism of carbohydrates. Porges[4] and later Bernstein[5] reported a lowering of blood sugar in Addison's disease, while Fuchs

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1. Blum: Deutsch. Arch. f. klin. Med., 1901, **71,** 146; and Arch. f. d. ges. Physiol. (Pfluger's), 1902, **90,** 628.

2. Bernard, Claude: Leçons de physiologie experimentelle au Collège de France, Paris, 1855.

3. Brown-Séquard: Compt. rend. Soc. de biol., 1889, pp. 415, 420, 430, 451.

4. Porges: Ztschr. f. klin. Med., 1909, **69,** 341.

5. Bernstein: Berl. klin. Wchnschr., 1911, **48,** 1874.

and Roth[6] found, after injections of epinephrin, a distinct rise in the respiratory quotient in patients with this disease. Porges[7] also showed that the blood sugar in adrenalectomized dogs regularly falls during the time that the animal survives. The experiments of Bierry and Mallosel[8] confirmed this observation. They found that the concentration of sugar in the blood, after extirpation of the adrenals, fell to one fifth or one half of the original amount and remained there. They also showed that these animals were more resistant to epinephrin injections than normal animals so far as the production of hyperglycemia is concerned. More will be said about these observations later on in discussing the phase of carbohydrate metabolism affected by the adrenal secretion. It is obvious, however, from what has been said, that the adrenal glands by means of their internal secretion, and in particular by the active principle of the medullary portion, play some part in carbohydrate metabolism. The possibility that this power to alter and in part control the metabolism of sugar bears some relation to the pathogenesis of diabetes mellitus is, of course, of the greatest importance, and one which has formed the chief stimulus to the investigations. With this possibility in mind we shall review the experimental work and hypotheses which have been offered to explain the mechanism by which the secretion of the adrenals affects the storage and combustion of carbohydrates.

But before entering on a consideration of this question it will be well to recall briefly the important facts in normal carbohydrate metabolism. Carbohydrate food enters the body by the alimentary tract in the form of sugars and starches. By the action of diastatic enzymes of the saliva and pancreatic secretion the latter are split into sugars. In the intestine, chiefly in the upper portion of the small intestine, the sugars are acted on by the enzymes of the intestinal mucosa, namely, invertase, maltase and lactase, and thereby converted into one or other of the monosaccharids. By far the largest portion of the ingested carbohydrate is absorbed in the form of dextrose, but levulose and galactose have also been demonstrated in the portal blood. In the liver, glycogen is formed from these sugars and stored within the hepatic cells, probably by the action of an enzyme (glycogenase?) which is present in the liver cells. This formation of glycogen, which depends chiefly on glucose, but also to a slight extent on levulose and galactose, is called glycogenesis. Whenever the cells of the body, especially the striated muscles, require glucose for heat or energy needs, the glycogen stored in the liver is broken down by the glycogenase, and glucose is discharged into the hepatic veins, and thus to the general circulation.

6. Fuchs and Roth: Ztschr. f. exper. Path. u. Therap., 1912, **10**, 187.
7. Porges: See Note 4.
8. Bierry and Mallosel: Compt. rend. Soc. de biol., 1908, **60**, 232.

This process by which glucose is formed from glycogen is termed glycogenolysis. The tissues of the body either burn the glucose thus brought to them by the blood stream, forming carbon dioxid and water as the end-products, or they store it as glycogen. So accurately adjusted is this mechanism by which the liver supplies the circulating blood, and, indirectly, the tissues, with glucose, according to the tissue requirements, that the concentration of the glucose of the blood remains practically constant, despite great variations in the quantities the tissues use. Normal well-fed dogs made to run on the treadmill almost to the point of exhaustion show little, if any, alteration in the glucose concentration of the blood, despite the enormous consumption by the muscular tissues entailed during such exercise. Under physiologic conditions the blood sugar is kept between 70 and 100 mg. per 100 c.c. of blood. But in order that these processes shall proceed in this way it is essential that the islands of Langerhans be functionating. Removal of the pancreas promptly upsets this mechanism; the blood sugar rises, glycosuria appears, the glycogen stores become impoverished, and the animal presents an essentially complete picture of diabetes mellitus.

Any disturbance in this mechanism for sugar control is promptly shown by changes in sugar concentration of the circulating blood. Overproduction by the liver, that is, hyperglycogenolysis, is at once followed by a rise in the sugar of the blood. Diminished or arrested consumption, that is, a disturbance in glycolysis, produces a similar result.

Following an injection of epinephrin there occurs some disturbance in the physiologic sequence of events just outlined, a disturbance manifested by the appearance of considerable quantities of sugar in the urine. One or more of the several phases of carbohydrate metabolism must, therefore, be affected either in rate or character. We must consider each of the possibilities: does epinephrin alter the process of sugar absorption from the intestine; or is it in the processes of formation or breaking down of glycogen that it intervenes; or does it affect the oxidation of the sugar; or does it act on the internal secretion of the pancreas, rendering it incapable of exerting its normal control of sugar metabolism; or, finally, does it simulate the action of phlorizin and lower the threshhold for sugar excretion by the kidneys?

There is, perhaps, one further possibility which, alone, could hardly account for the epinephrin effects, but as an ancillary factor, might play a part. This is glyconeogenesis, or the formation of glucose from protein. If epinephrin caused the breaking down of the protein molecule and the liberation of the carbohydrate fraction, this could increase the effects of such a carbohydrate disturbance as epinephrin produces; but with glycogenic, glycogenolytic and glycolytic processes proceeding

normally, glyconeogenesis could not explain the metabolic changes following an increase of the circulating epinephrin. Such an increased production of carbohydrate would be quite comparable to carbohydrate feeding in a normal animal — the excess would either be stored as glycogen or oxidized.

It is not necessary to discuss the possibility that epinephrin affects the process of sugar absorption by the intestines; suffice it to say that there is neither theoretical reason nor experimental evidence pointing to any such action.

We shall, therefore, proceed to examine the experimental data and the conclusions which may be drawn from them in so far as they throw light on the remaining theoretically possible explanations of the mechanism of the epinephrin action.

Blum's results were promptly confirmed (Zuelzer,[9] Metzger,[10] Herter and Richards,[11] Paton,[12] Lazarus[13]), and it soon became well established that the injection of 1.0 mg. of epinephrin per kilo of body weight is followed by a glycosuria which appears in one half to two hours, and may last a few hours to a day or longer. Moreover, it does not matter whether a saline extract of the fresh gland be employed or one of the pure products, such as the adrenalin chlorid (Aldrich,[14] Takamini[15]), the epinephrin of Abel,[16] adrenin, or the synthetic product.[17]

First Zuelzer[9] and then Metzger[10] and Paton[12] showed that the glycosuria is accompanied by an hyperglycemia, thus ruling out the possibility that the mechanism is similar to that of phlorizin diabetes, in which, despite glycosuria, there is hypoglycemia, and probably a lowering of the threshhold for sugar excretion by the kidney. Vosburg and Richards[18] obtained, after epinephrin injections, a more marked hyperglycemia in well fed animals than in those which had been starved, suggesting thus that the glycogen stores had been called on to give up glucose to the blood. Pollak[19] showed that both hyperglycemia and glycosuria are more readily produced by subcutaneous than by intravenous or intraperitoneal administration. He explains this by the assumption that the carbohydrate mechanism is more sensitive to epinephrin than the vasoconstrictors, and that the slow absorption after subcutaneous injections allows the epinephrin concentration of the blood to rise only to a level at which the carbohydrate mechanism is

9. Zuelzer: Berl. klin. Wchnschr., 1901, **38,** 1209.
10. Metzger: München. med. Wchnschr., 1902, **12,** 478.
11. Herter and Richards: Med. News, New York, 1902, **80,** 201.
12. Paton: Jour. Physiol., 1903, **29,** 286.
13. Lazarus: Cited by Shafer, Brit. Med. Jour., 1908, **1,** 1277.
14. Aldrich: Am. Jour. Physiol., 1901, **5,** 457.
15. Takamini: Am. Jour. Pharm., 1901, **11,** 523.
16. Abel: (Hoppe-Seyler's) Ztschr. f. Physiol. Chem., 1899, **28,** 318.
17. Amberg: Arch. Intern. de Pharmakol., 1902, **11,** 57.
18. Vosburg and Richards: Am. Jour. Physiol., 1903, **9,** 35.
19. Pollak: Arch. f. exper. Path. u. Pharmakol., 1909, **61,** 149.

affected, while the more rapid entrance of the epinephrin into the circulation after intravenous or intraperitoneal injection raises the concentration in the blood sufficiently high to cause vasoconstriction. Consequently a diminished amount of blood passes through the liver, and thus less glucose is mobilized. Straub[20] and Ritzmann,[21] on the basis of infusion experiments, have shown that the glycosuria runs almost parallel with the epinephrin concentration of the blood, and Ritzmann

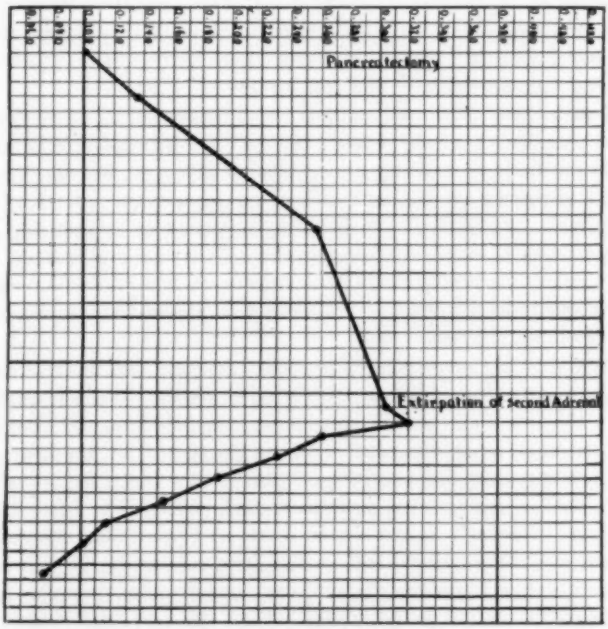

CHART 1.—Blood sugar curve in depancreatized dog, with extirpation of adrenals. Ordinates = per cent. reducing substance in blood. Abscissa = time: Each square = two hours.

puts forward the idea that epinephrin acts to maintain, by sympathetic stimulation, a *Zuckertonus* as well as a *Gefässtonus*. Underhill's[22] experiments with epinephrin infusions corroborate Ritzmann's results. Satisfactory investigations as to the parallelism between sugar and epinephrin concentrations in the blood are lacking.

Of considerable importance in determining how and where epinephrin acts is, first, the question of the effect of epinephrin injections on the glycogen content of the liver, and, second, whether or not it is

20. Straub: München. med. Wchnschr., 1909, **56,** 493.
21. Ritzmann: Arch. f. exper. Path. u. Pharmakol., 1909, **61,** 231.
22. Underhill: Jour. Biol. Chem., 1911, **9,** 13.

still capable of causing hyperglycemia and glycosuria after the glycogen depots of the body have been exhausted.

The first of these questions has been definitely settled. Doyon and Kareff,[23] Gatin-Gruzewska,[24] Agadschanianz[25] and Pollak[26] have shown that epinephrin diminishes the quantity of glycogen in the liver. Schirokauer and Wilenko[27] went a step further and showed that the increased glycogenolysis in animals treated with epinephrin is not dependent on an increased production of liver diastase. Furthermore, Gatin-Gruzewska[24] and Agadschanianz[25] demonstrated that the muscles also lose glycogen. So far as I am aware there are no trustworthy experiments contradicting this conclusion.

If the second of these questions could be answered affirmatively, it would constitute a strong support for the view that epinephrin acts not merely on the liver, causing it to discharge glycogen, but also that it is capable of raising the concentration of the blood sugar, either by interfering with the glycolytic processes in the tissues or by inducing glyconeogenesis, or by inhibiting the pancreas. It becomes necessary, therefore, to examine more closely the evidence on which conclusions have been based as to the effect of epinephrin on animals whose livers have been rendered glycogen-free.

Blum,[1] in his early communications, asserted that epinephrin glycosuria occurred even when the glycogen stores had been exhausted before the injection, but his conclusion was based on the assumption that starvation alone will remove all the glycogen from the liver. This is open to question, because Pflüger[28] has shown that a dog after starving twenty-eight days still contains enough glycogen to form 100 gm. of sugar. Ringer[29] found no increase in glycosuria following epinephrin injections after the liver had been rendered glycogen-free by phlorizin. This, however, is not convincing proof, because little is known of the action of phlorizin, beyond the fact that it lowers the threshhold for sugar excretion by the kidney. It may also interfere with the mechanism involved in epinephrin glycosuria. Paton[12] and Herter and Richards[11] offered evidence in support of Blum. They found that dogs which had been either fasted or fed on a diet of material from which no glycogen could be formed and then phlorizined, still responded with glycosuria to epinephrin injections. It seems doubtful, however, that the animals were glycogen-free before the injection. Frank and Isaac[30] found that after dogs were poisoned with phos-

23. Doyon and Kareff: Compt. rend. Soc. de biol., 1904, **56,** 66.
24. Gatin-Gruzewska: Compt. rend. Acad. de sc., **142,** 1165.
25. Agadschanianz: Biochem. Ztschr., 1907, **2,** 148.
26. Pollak: Arch. f. exper. Path. u. Pharmakol., 1909, **61,** 153.
27. Schirokauer and Wilenko: Ztschr. f. klin. med., 1910, **70,** 257.
28. Pflüger: Arch. f. d. ges. Physiol., 1902, **91,** 119.
29. Ringer: Jour. exper. Med., 1910, **12,** 105.
30. Frank and Isaac: Ztschr. f. exper. Path. u. Therap., 1910, **7,** 326.

phorus no hyperglycemia followed epinephrin injections. Phosphorus is known to cause a disappearance of the liver glycogen, but the absence of epinephrin glycosuria during such a profound metabolic disorder as phosphorus causes does not settle the question. Velich[31] found no epinephrin glycosuria after extirpation of the liver in frogs. Falta and

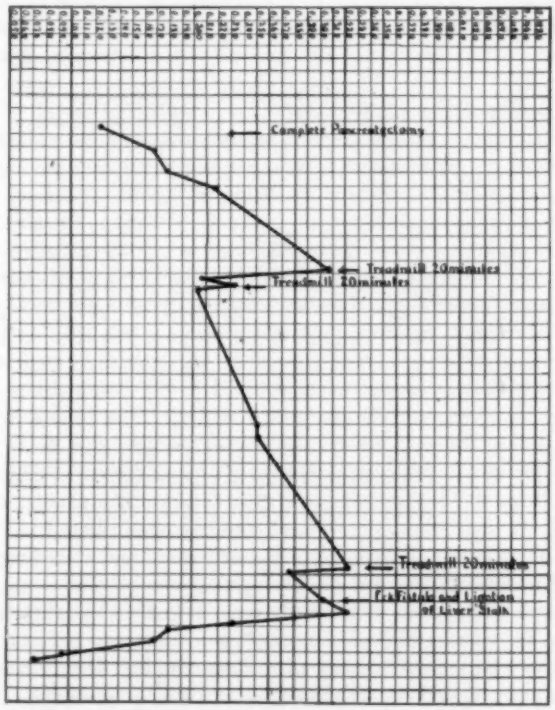

CHART 2.—Blood sugar curve in depancreatized dog with Eck fistula and ligation of liver stalk. (The first portion of this curve was made for another experiment in which the effect of exercise on the blood sugar was being studied.) The rapid and uninterrupted drop in the amount of reducing substance in the blood after the liver was excluded from the circulation is very striking. Ordinates represent per cent. of reducing substance in the blood. Abscissa = time: Each square = two hours.

Priestly,[32] using rabbits, tied off the liver vessels and found that, despite epinephrin injections, the blood sugar fell to 0.04 per cent. The conclusion from this seems to be that the liver glycogen is essential for the maintenance of the blood sugar level, and that the glycogen stores

31. Velich: Virchows Arch. f. path. Anat., 1906, **184,** 345.
32. Falta and Priestley: Berl. klin. Wchnschr., 1911, **48,** 2102.

in the muscles are inadequate for this. Michaud's[33] experiments on dogs with Eck's fistulas are important. In only one out of six dogs did epinephrin cause a rise in blood sugar. His animals remained in good condition after the operative interference, a condition which has usually been lacking in experiments aimed to settle this point, and one which has doubtless contributed to the contradiction of results. Pollak[34] induced convulsions with strychnin in starved rabbits, presumably glycogen-free. Although it did not produce hyperglycemia, epinephrin did cause a disturbance in carbohydrate metabolism, shown by the deposition of glycogen in the liver.

It is not easy to give a definite interpretation of the experimental data mentioned; experiments of many types have been performed to determine whether the glycosuric and hyperglycemic action of adrenal persists without available glycogen in the liver, but, as the above mentioned investigations show, there has been by no means a uniformity in the results. Perhaps the experiments of Pollak[36] and Michaud[33] are the most significant; from them we may conclude, with some reserve, however, that an available supply of glycogen in the liver is essential for the production of hyperglycemia by epinephrin injections, and, furthermore, that an excess of epinephrin in the blood stimulates the formation of glycogen in a glycogen-free liver. But there are other reasons, besides those advanced by Pollak, which lead us to believe that epinephrin has some influence on glycogen formation, as well as on glycogenolysis. Schwarz,[35] using rats, because they survive adrenalectomy for a long time, determined the glycogen content (Pflüger's method) after both adrenals had been extirpated, and found only mere traces or complete absence of glycogen. He concludes that after adrenalectomy the power to store glycogen is lost. This work has been confirmed by Kahn and Starkenstein,[36] who not only demonstrated a loss of liver glycogen following adrenalectomy, but demonstrated also that such animals acquire an unusual tolerance for epinephrin.

I have administered large quantities of glucose intravenously and by the alimentary tract to adrenalectomized dogs, and have found that when, by thus saturating the organism with sugar, the concentration of sugar in the blood has been raised to 560 mg. per 100 c.c., little or no glycogen is demonstrable in the liver cells when stained by Best's method. Control animals which have been starved, but not adrenalectomized, receiving the same amounts of sugar per kilo body weight, show abundant glycogen granules in the liver cells. Since we know that an excess of epinephrin in the blood causes a discharge of gly-

33. Michaud: Verhandl. d. deutsch. Kongr. f. inn. Med., Wiesbaden, 1911, p. 561.
34. Pollak: Arch. exper. Path. u. Pharmakol., 1909, **61,** 166.
35. Schwarz: Arch. d. ges. Physiol. (Pflüger's), 1910, **134,** 259.
36. Kahn and Starkenstein: Arch. f. d. ges. Physiol. (Pflüger's), 1911, **139,** 181.

cogen from the liver, the observation that a diminished amount of epinephrin in the blood is associated with an absence of glycogen, carries with it the conclusion that epinephrin performs a double function, so far as glycogen metabolism in the liver is concerned. It is necessary for glycogenesis, and when present in excess it stimulates glycogenolysis. Or, stated in another way, when it is absent, glycogen storage is inhibited; when it is present in excess, the transformation of glycogen to glucose is accelerated.

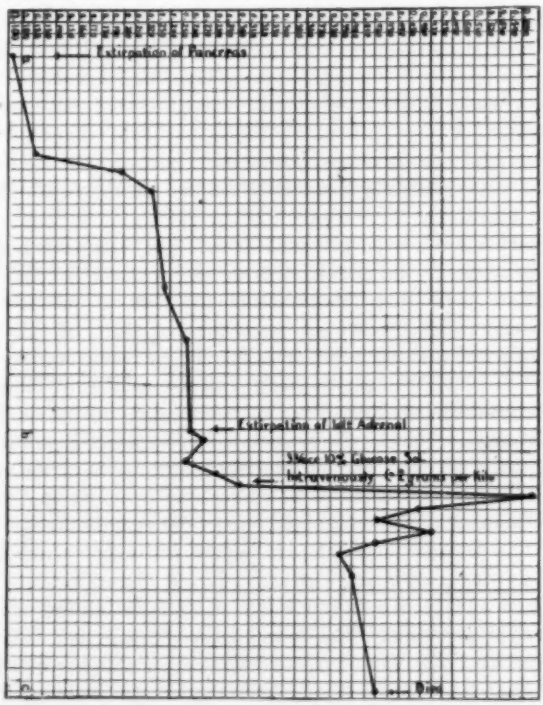

CHART 3.—Blood sugar curve in depancreatized dog after extirpation of the adrenals, followed by intravenous injection of glucose solution—2 gm. per kilo. Ordinates = per cent. of reducing substance in blood. Abscissa = time: a-b each square = two hours; b-c each square = one-half hour.

Having now satisfactory evidence that the internal secretion of the adrenals intervenes in carbohydrate metabolism in these two ways, we must proceed to examine the data which throw light on the chemical or physical processes by which these functions are performed. We can imagine that such a mechanism is dependent on direct hormone action within the liver cells, the epinephrin acting, on the one hand, on the glucose brought by the portal blood, converting it to glycogen, and, on

the other hand, on the glycogen of the liver, converting it to glucose. Or we can think of it as producing its effects by controlling the blood supply of the liver, the changes in the condition of the carbohydrate in the liver being then dependent on the constriction or dilatation of the blood vessels. We may also, as some investigators hold, believe that its action depends on a specific power to affect the internal secretion of the pancreas. Finally, if it could be shown that glycogenesis and glycogenolysis are controlled by nervous impulses carried by the sympathetic, the well known stimulating effects of epinephrin on sympathetic nerve endings might conceivably explain the mechanism.

That there is an intimate relation between the nervous system and the sugar content of the blood has long been well known. Sixty-one years ago Claude Bernard[2] published the results of his observations on the appearance of glycosuria following puncture of the floor of the fourth ventricle. Many investigators have been interested since then in studying experimentally the mechanism by which piqûre glycosuria is brought about.

Naunyn[37] found a hyperglycemia of 0.81 per cent. four hours after piqûre. Because of the inexactness of the methods of blood sugar determination, these early observations are open to question. Bang, Ljungdahl and Bohm,[38] however, found a blood sugar of 0.38 per cent. after piqûre in rabbits, and this has been confirmed by others. Eckhardt[39] and later Pflüger[40] agreed with the opinion of Bernard that the stimuli produced by piqûre are carried directly to the liver by way of the splanchnics. Blum's[1] discovery of epinephrin glycosuria, however, suggested at once that epinephrin might be a factor in piqûre glycosuria. That the secretion of epinephrin is under the control of the sympathetic has been well established (Asher,[41] Popielski,[42] Tscheboksareff[43]). Pollak[44] showed that piqûre and other centrally acting causes of hyperglycemia are ineffective if the splanchnics are severed, and Macleod and Pearce,[45] by electric stimulation of the splanchnics, produced hyperglycemia. The results of Waterman and Smit,[46] who, using the Meltzer-Ehrmann reaction as a test for increase of epinephrin in the blood, claimed to have demonstrated an increase following piqûre, have met with some opposition. Later researches by Kahn,[47]

37. Naunyn: Arch. f. exper. Path. u. Pharmakol., 1874, **3,** 85.
38. Bang, Ljungdahl and Bohm: Beitr. z. chem. Physiol. u. Path. (Hofmeister), 1907, **10,** 1.
39. Eckhardt: Beitr. z. Anat. u. Physiol., 1869, **4,** 4, 138.
40. Pflüger: Das Glykogen, Ed. 2, 1905.
41. Asher: Centralbl. f. Physiol., 1910, **137,** 927.
42. Popielski: Arch. f. d. ges. Physiol., 1911, **139,** 571.
43. Tscheboksareff: Centralbl. f. Physiol., 1910, **137,** 137.
44. Pollak: Arch. f. exper. Path. u. Pharmakol., 1909, **61,** 376.
45. Macleod and Pearce: Am. Jour. Physiol., 1912, **29,** 419.
46. Waterman and Smit: Arch. f. d. ges. Physiol. (Pflüger's), 1908, **124,** 198.
47. Kahn: Arch. f. d. ges. Physiol. (Pflüger's), 1912, **144,** 251 and 396.

Lopez,[48] and v. Brucke[49] failed to confirm the findings of Waterman and Smit. It should be remembered that the tests for epinephrin in the blood are not as precise as could be desired[50] and the results reported should not be too readily accepted. There are four more or less reliable physiologic tests for the presence of minute quantities of epinephrin in the blood: (1) its mydriatic (dilatory) action on the pupil of the excised frog's eye; (2) its effect in raising the arterial blood pressure; (3) its power of lessening the rhythmic contractions of the longitudinal coat of the intestine immersed in Ringer's solution; (4) its stimulating action on the rhythmical contractions which occur in the excised uterus of the virgin rabbit in oxygenated Ringer's solution. The reason for caution in interpreting such results is that serum may contain substances which stimulate and substances which inhibit[51] the action of epinephrin. Borberg,[52] using an improved technic for the Meltzer-Ehrmann reaction, found a marked rise in the adrenalin content of the adrenal vein after diabetic puncture. Kahn,[53] moreover, on the basis of indirect evidence, believed that piqûre caused a discharge of epinephrin. He found a decrease of chromaffin substance in the adrenals after piqûre, and assumed from this that there was an increased secretion. Jarisch[54] was unable to confirm this. Trendelenburg and Fleischhauer[55] offered further indirect evidence against the view that piqûre is followed by an increased concentration of epinephrin in the blood. They determined the amount of epinephrin which, by continuous intravenous infusion, would produce a glycosuria equivalent to that following piqûre, and observed that when such amounts of epinephrin were in the blood there was at the same time a marked rise in the blood pressure. Since this is absent after piqûre, they believe that an hyper-adrenalinemia is not the essential factor in piqûre glycosuria.

Further evidence as to the rôle of the adrenals in the production of piqûre hyperglycemia and glycosuria is found in the numerous experiments on the effect of piqûre or splanchnic stimulation after extirpation of the adrenals. Mayer[56] did piqûre on twenty-five adrenalectomized rabbits and failed to produce glycosuria. Landau[57] confirmed this. Kahn[58] found that after extirpation of the adrenals rabbits showed no glycosuria. In later experiments,[58] however, he found

48. Lopez: Arch. f. d. ges. Physiol. (Pflüger's), 1912, **145,** 311.
49. Von Brucke: München. med. Wchnschr., 1911, p. 1389.
50. Macleod: Diabetes, London, 1913.
51. Stewart: Jour. Exper. Med., 1911, **14,** 377.
52. Borberg: Dissertation, Kopenhagen, 1912.
53. Kahn: Arch. f. d. ges. Physiol. (Pflüger's), 1911, **140,** 209.
54. Jarisch: Ztschr. f. exper. Path. u. Therap., 1913, **13,** 520.
55. Trendelenburg and Fleischhauer: Ztschr. f. d. ges. exper. Med., 1913, **1,** 369.
56. Mayer: Compt. rend. Soc. de biol., 1906, **60,** 1123.
57. Landau: Experimentelle Nebennieren-Studien, Dorpat, 1908.
58. Kahn: Arch. f. d. ges. Physiol. (Pflüger's), 1909, **128,** 519.

that piqûre causes a loss of chromaffin substance from the adrenals, and from this concluded that piqûre stimulates the secretion of epinephrin. Freund and Marchand[59] found that piqûre in adrenalectomized animals is followed by marked hyperglycemia, but they found no sugar in the urine. They concluded that its hyperglycemic action does not depend on the adrenals, but results from direct stimulation of the liver. Wertheimer and Battez[60] found that dogs and cats exhibit a difference in their reaction to piqûre. Adrenalectomized cats showed a marked glycosuria (3.63 to 17.5 gm.) after piqûre, but dogs on which they performed similar experiments, showed none. Gautrelet and Thomas[61] stimulated the splanchnics after extirpation of the adrenals and found no glycosuria.

That the blood sugar curves[62] after epinephrin injections and after piqûre are different affords some evidence against the view that the mechanism is similar, but the great variability of the curves after piqûre makes deductions from this hazardous.

Perhaps the most illuminating experiments dealing with this problem are those of Macleod[63] and his collaborators. Realizing that the disturbances of respiration and circulation following piqûre vitiate, in part at least, the hyperglycemic effects of this procedure, he demonstrated that the cava blood contains an increased quantity of reducing substance after stimulation of the great splanchnic nerve with the adrenals intact. After excision of the gland on the left side, stimulation of the nerve on the same side causes no rise in the blood sugar. Gautrelet and Thomas[61] had obtained results similar to these, but since this did not prove that the hyperglycemia was due to an increased secretion of epinephrin, Macleod went further and elaborated a type of experiment first done by Kaufmann.[64] He sectioned the nerve path between the adrenals and the liver, that is, the hepatic plexus. When this was done he found that stimulation of the left splanchnic was only rarely followed by an increase in the sugar of the blood, and that when this occurred it was less intense than usual. The conclusion from this is that it cannot be merely an increased epinephrin secretion which causes the hyperglycemia. He then applied electrical stimulation directly to the hepatic plexus and found that when the adrenals were intact such stimulation caused a striking hyperglycemia, whether the fibers central to the point of stimulation were cut or uncut. In the absence of the adrenal glands, however, stimulation of the hepatic plexus had no effect. The conclusion arrived at from these experi-

59. Freund and Marchand: Arch. f. exper. Path. u. Pharmakol., 1914, **76,** 324.
60. Wertheimer and Battez: Compt. rend. Soc. de biol., 1914, **86,** 617.
61. Gautrelet and Thomas: Compt. rend. Soc. de biol., 1909, **67,** 233.
62. Bang: Der Blutzucker, Wiesbaden, 1913, p. 113.
63. Macleod: Diabetes, London, 1913, and Macleod and Pearce: Am. Jour. Physiol., 1912, **29,** 419.
64. Kaufmann: Arch. de Physiol., 1895, **27,** 266.

ments is that only when the adrenal glands are intact is it possible, by stimulation of the nerves supplying the liver, to excite hyperglycogenolysis and hyperglycemia. Some influence exercised by the adrenal glands is evidently essential for the functional integrity of the nerves which control the process of glycogenolysis. That this rôle of activating the receptive substance which, according to the experiments of Elliott[65] and others, is believed to lie between the nerve terminations and the cell substance, is not out of harmony with the fact that an excess of epinephrin in the blood causes an increase of glycogenolysis, is shown by other experiments of Macleod. Hyperglycemia was produced by injecting epinephrin into the portal vein after the sympathetic stimuli had been stopped by cutting the hepatic plexus. This explanation of the mechanism by which epinephrin produces its glycosuric effect has recently been substantiated by the work of Freund.[66] Taking advantage of the fact that rabbits have a collateral liver circulation through diaphragmatic vessels which is sufficient to prevent necrosis when the hepatic artery is tied, he cut the hepatic artery and hepatic plexus before doing piqûre. His results were not entirely constant, but on the whole indicated that piqûre does not cause hyperglycemia unless the nerve path between adrenals and liver is intact. Hence piqûre hyperglycemia is not dependent on an increase of epinephrin in the blood.

The possibility that the internal secretion of the adrenals interferes with the pancreatic control of carbohydrate metabolism by a specific inhibitory action, is an attractive theory, but one for which sound experimental proof is still lacking. In the first place it is not yet clear just what rôle in carbohydrate metabolism is played by the pancreas. The secretion which performs this function has never been isolated. The effects of removal of the gland are well known, but just how it acts when present, it is impossible to say. From the work of Hédon[67] and later that of Lombroso,[68] MacCallum[69] and Forschbach[70] it may be accepted as an established fact that the pancreas, independent of its external secretion, and of the nerve connections, performs an important function in the mobilization or utilization of glucose, and that probably this is accomplished by an internal secretion. Investigators are divided on the question of whether or not the hyperglycemia and glycosuria of pancreatic insufficiency are due to a primary underconsumption or a primary overproduction, and it is not necessary for our present purpose to discuss this question, except insofar as it

65. Elliott: Compare Swale Vincent: Ergebn. der Physiol., 1910, **9,** 451.
66. Freund: Arch. f. exper. Path. u. Pharmakol., 1914, **76,** 311.
67. Hédon: Compt. rend. Soc. de biol., 1890.
68. Lombroso: Ergebn. der Physiol., 1910, **9,** 1.
69. MacCallum: Bull. Johns Hopkins Hosp., 1909, **20,** 265.
70. Forschbach: Deutsch. med Wchnschr., 1908, **34,** 910; Arch. f. exper. Path. u. Pharmakol., 1908, **60,** 131.

involves the theory advanced by Eppinger, Falta and Rudinger[71] regarding the specific interaction of the adrenals and pancreas. They found that in depancreatized dogs injections of epinephrin caused an increase in the sugar excretion, and a rise of the D:N ratio, but the objection has been made that they offer no proof that the diabetes was complete before the epinephrin was injected. Ringer[29] failed to produce an increase in the glycosuria of fully phlorizinized dogs by epinephrin injections. Zuelzer's[72] observations that injections of pancreas extract rendered epinephrin nonglycosuric seemed to support the view that after pancreatectomy the diabetic manifestations appear because the internal secretion of the adrenal is then permitted, free from pancreas opposition, to act as a stimulus on the sugar output of the liver; but it was later shown that[73] many irritating substances when injected intraperitoneally have the same inhibitory effect on epinephrin glycosuria. Frugoni,[74] however, found that the inhibitory effect is obtained when the extract and epinephrin are injected subcutaneously. Loewi found that after removal of the pancreas the serum has an increased mydriatic power, and believes that this is due to the action of the epinephrin in the serum from which the inhibiting pancreatic secretion has been removed. He found human diabetic patients more susceptible to epinephrin mydriasis than normal controls.

Biedl and Offer[75] found that the lymph from the thoracic duct contains something which antagonizes the action of epinephrin on the excised frog's eye, and also inhibits epinephrin glycosuria. Believing this substance to be the internal secretion of the pancreas, they support the view of a mutually inhibitory action by the pancreas and adrenals.

A few experiments on the effect of adrenal extirpation on pancreatic diabetes in animals have been attempted. The difficulty here has been that the animals die so soon after removal of the adrenals that conclusions drawn from blood sugar determinations while the animals were in a moribund state are untrustworthy. Frouin[76] extirpated about three quarters of the adrenal substance from animals which had previously been rendered diabetic by removal of the pancreas. He found that the intensity of the diabetes was decreased as compared with controls. Mayer[77] destroyed by cauterization the adrenals of depancreated dogs, but the animals survived the operation only an hour. In experiments on cats he removed the adrenals in two stages, and found that they then lived two to five hours after the operation. In each of three cases there was a fall in the blood sugar.

71. Eppinger, Falta and Rudinger: Ztschr. f. klin. Med., 1909, **66,** 1.
72. Zuelzer: Berl. klin. Wchnschr., 1909, **46,** 1209.
73. Von Fürth and Schwarz: Biochem. Ztschr., 1911, **31,** 113.
74. Frugoni: Berl. klin. Wchnschr., 1908, **45,** 1606.
75. Biedl and Offer: Wien. klin. Wchnschr., 1907, **20,** 1530.
76. Frouin: Compt. rend. Soc. de biol., 1908, **60,** 216.
77. Mayer: Compt. rend. Soc. de biol., 1908, **60,** 219.

I have studied the effect of adrenal extirpation in the following way: The right adrenal, being the more difficult of the two to remove, is extirpated at the first operation. The animal is then allowed a week or more in which to make a complete recovery from the operation. Such dogs show no signs of adrenal insufficiency. At the second operation the pancreas is removed. When the blood sugar has risen to a diabetic level the left adrenal is extirpated. This is an easy operation, and can be done through a lumbar incision in twenty minutes. During the time that the animal survives, frequent blood sugar determinations are made, and the results plotted in a curve. Such an experiment on a dog is represented in Chart 1. It will be seen that following removal of the pancreas the blood sugar rose rapidly from the normal level (0.100 per cent.) to 0.305 per cent. Then the second adrenal (left) was removed. After a short rise, doubtless due to nerve stimulation during the operation, the blood sugar rapidly and uninterruptedly fell to normal. The dog survived the operation twenty-three hours, and during the first five or six hours after recovery from the anesthetic remained in good condition. This experiment has been repeated several times with the same result. The complete results of these experiments will shortly be published.

We see, therefore, that a considerable mass of evidence has accumulated to support the theory of a specific interaction between the adrenals and pancreas. None of it, however, is completely convincing. We shall revert to this question later on, after having considered the possibility that the adrenal secretion affects the power of the tissues, particularly of the muscles, to utilize sugar.

But first the explanation offered by Edmunds[78] and recently supported by the work of Mann and Drips[79] must be mentioned. Benedicente[80] and Pemberton and Sweet[81] believed that because there is a diminished flow of the external secretion of the pancreas following epinephrin injections, there was also an inhibition of the secretion of the islands of Langerhans. Edmunds, however, offered evidence in support of the view that the diminished pancreatic secretion is merely consequent on the vasoconstriction produced by epinephrin. The increased flow after adrenalectomy is due according to Mann and Drips merely to the changes in blood pressure, decreased temperature, etc., incident to the moribund condition. It is, therefore, clear that before the specific relationship between islands of Langerhans and adrenal medulla be accepted, the effect of alterations in the blood supply of the pancreas after adrenalectomy or epinephrin injections must be excluded.

78. Edmunds: Jour. Pharm. and Exper. Therap., 1911, **2**, 559.
79. Mann and Drips: THE ARCHIVES INT. MED., 1915, **16**, 681.
80. Benedicente: Arch. d. biol., 1905, **95**, 1.
81. Pemberton and Sweet: THE ARCHIVES INT. MED., 1908, **1**, 628.

If it could be shown that the suprarenal secretion possesses the power to inhibit the oxidation of sugar by the tissues, it would strongly support the view, which has here and there cropped out in the literature of the subject, that in diabetes the basic fault is an excess of secretion by the adrenals or a diminished inhibition of this secretion by the pancreas. Experiments on the respiratory quotient and heat production supply the most significant facts in determining whether or not epinephrin affects glycolysis.

The respiratory quotient represents the ratio of the amount of carbon dioxid formed to the amount of oxygen required in the combustion of foodstuffs. Glucose, having sufficient oxygen to combine with all the hydrogen present to form water, will require for every molecule of carbon dioxid formed one molecule of oxygen. Plainly, then, the glucose respiratory quotient (R. Q.) is 1. In the case of protein the R. Q. is 0.81. Fat contains relatively less oxygen and consequently has a lower R. Q. This has been found to be 0.70. When, therefore, an animal is burning carbohydrate the R. Q. will be high; if he is burning only protein or protein and fat it will fall somewhere between 0.70 and 0.81.

Wilenko[82] studied the influence of sugar ingestion and epinephrin administration in rabbits under urethane anesthesia. He found (1) that epinephrin has little or no effect on the R. Q. of fasting rabbits, the carbohydrate stores having presumably been exhausted; (2) that epinephrin depresses the physiologic elevation of the R. Q. after administration of carbohydrate; (3) that intravenously administered glucose in epinephrin animals appears quantitatively in the urine. His conclusion is that epinephrin diminishes the ability of the organism to burn sugar. La Franka[83] had previously reported experiments in which he found a lowering of the R. Q. in phlorizin and pancreas glycosuria, but no change after epinephrin administration. Hári[84] starved dogs twenty-four to thirty-six hours, and then after curarizing them, injected epinephrin intraperitoneally and intravenously. He found that the epinephrin increased the R. Q., indicating that more sugar was burned after the injections than before. Fuchs and Roth,[8] in two cases of Addison's disease, found a distinct rise in the R. Q. after subcutaneous epinephrin injections. Similar results have been reported by Falta.[85] Lusk[86] has attempted to settle this question by experiments with the respiration calorimeter. He found that epinephrin administered twenty-one hours after food ingestion does not prevent the oxidation of glucose in a well-nourished dog. He also injected epi-

82. Wilenko: Biochem. Ztschr., 1912, **42**, 44.
83. La Franka: Ztschr. f. exper. Path. u. Therap., 1909, **6**, 1.
84. Hári: Biochem. Ztschr., 1911, **38**, 23.
85. Falta: Die Erkrankungen der Blutdrusen, 1913, p. 428.
86. Lusk: THE ARCHIVES INT. MED., 1914, **13**, 673.

nephrin during a period of glucose absorption, and found no depression of the R. Q. following the injections. During two experimental periods of six hours and five hours, respectively, he found the R. Q. to be 0.98 and 0.99.

The preponderance of evidence, therefore, so far as alteration in the respiratory quotient is concerned, favors the view that epinephin does not inhibit the combustion of sugar. Efforts[87] to show that epinephrin can act as an antiferment, inhibiting the action of the glycolytic ferment of the blood, have been unsuccessful.

The observations cited above that the hyperglycemia in experimental pancreas diabetes rapidly disappears after extirpation of the adrenals, or is prevented by simultaneous adrenalectomy, have led to the assumption that in the absence of both pancreas and adrenals the animal is able to oxidize glucose. This is almost equivalent to saying that when the blood sugar of such an animal has fallen to a normal level his diabetes has been cured, and that death comes from adrenal insufficiency, even though the power of carbohydrate combustion has been restored. Such an assumption is gratuitous, because the mechanism regulating the mobilization of sugar is susceptible to so many influences — nervous, circulatory and chemical — that results after extirpation experiments are not convincing. In depancreatized dogs I have observed a return of the blood sugar to normal levels when an Eck fistula was created, and the stalk of the liver ligated. Chart 2 represents the blood sugar curve in such an experiment. The drop in the blood sugar on exclusion of the liver from the circulation is exactly similar to that produced by adrenal extirpation. Further evidence against the antiglycolytic action of epinephrin and the view that adrenalectomy restores to the animal with pancreas diabetes the power to burn sugar, is supplied by the experiment represented in Chart 3. In this experiment the right adrenal was extirpated on December 29. This produced no change in the blood sugar. One month later, after the animal had made a complete recovery, and seemed to be quite well, the pancreas was removed. Three days later the blood sugar was 260 mg. per 100 c.c. The left adrenal was then removed, and 2 gm. of glucose per kilo were injected intravenously. The blood sugar immediately shot up to 560 mg. per 100 c.c. If this animal had had his power of sugar consumption restored he should have shown a steady fall in the blood surgar curve, but this did not occur. The blood sugar took a sharp drop to a level somewhat above the level before the sugar injection and remained there until he died. The sugar excreted in the urine during the last twenty-four hours was 101.8 gm., while during the preceding twenty-four hours it had been 64.9 gm. Since 33.6 gm.

87. Mackenzie: Jour. Exper. Med., 1915, **22**, 757.

were injected it is evident that the intravenously administered glucose was recovered quantitatively in the urine, and there is no evidence that any sugar was burned. Moreover, sections of liver and striated muscle taken immediately after death, fixed in absolute alcohol and stained by Best's picrocarmin method, showed no evidence of glycogen. One may conclude from this that not only was there no restoration of glycolytic power, but also no return of the power to store glycogen after extirpation of the adrenals.

It is evident from this review of the experimental work designed to clear up the problems of the relation of the adrenal secretion to glycolysis, that the weight of evidence favors the view that they are unrelated. Wilenko's experiments on the respiratory quotient changes after epinephrin injections have not been confirmed by other investigators, and Zuelzer's conclusion, founded on a disappearance of reducing substance from the blood after adrenal extirpation, is based on insufficient evidence. We must conclude, therefore, that epinephrin does not interfere with the process of sugar oxidation by the tissues.

CONCLUSIONS

To conclude, it seems that we are justified, from the work that has been done, in accepting the following view of the relation of the suprarenal system to carbohydrate metabolism:

1. Nervous stimuli, especially of the sympathetic, represented by piqûre or splanchnic stimulation, are followed by an increased secretion of epinephrin, and this hyperadrenalinemia like that following epinephrin injections, causes hyperglycemia and glycosuria in part by inhibiting glycogenesis, and in part by furthering glycogenolysis.

2. The hyperglycogenolysis thus produced is dependent partly on a direct stimulation of the liver cells and partly on its action in rendering the receptive material between the sympathetic nerve endings and the liver cells more sensitive to nervous stimulation.

3. Epinephrin does not produce its effects by inhibiting glycolysis, and the disturbances in sugar metabolism following its administration have little or nothing to do with the loss of glycolytic power, which is probably a part of the altered metabolism in diabetes mellitus.

4. A specific physiologic relation between the islands of Langerhans and the adrenal medulla is unproved.

5. Adrenalectomized dogs show a diminution of the power to form glycogen from glycose.

6. Following extirpation of the adrenals in depancreatized dogs there is a rapid disappearance of hyperglycemia.

7. Sugar administered to such animals is neither oxidized nor stored as glycogen, but appears quantitatively in the urine.

A STUDY OF ETHYLHYDROCUPREIN (OPTOCHIN) IN THE TREATMENT OF ACUTE LOBAR PNEUMONIA *

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SYNOPSIS

Historical.
Scope of the problem and general considerations.
Character of the cases and their general treatment.
Technic employed; interpretation of results.

Dosage of optochin:

I. Oral administration entirely.
The various methods of dosage used; comparison.
Relationship between body weight of patient and appearance of bactericidal action in the serum.

II. Combined intramuscular and oral administration.

Absorption and elimination.
Fastness.
Toxic effects.

Influence on the clinical course of the disease:

1. Occurrence of "spreads."
2. Pneumococcal septicemia.
3. Duration of the disease.
4. Mortality rate.

Discussion.
Conclusions.

HISTORICAL

The valuable studies of Morgenroth and his co-workers[1] on the chemotherapy of the quinin alkaloids have shown that ethylhydrocuprein (optochin) has a specific bactericidal effect on the pneumococcus in vitro, and that this compound exerts a protective and curative action in animals experimentally infected with virulent strains of that micro-organism. The specific germicidal action of ethylhydrocuprein on the pneumococcus is greater than that of any other compound of the series examined, namely, quinin, hydroquinin (methylhydrocuprein),

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* From the Hospital of The Rockefeller Institute for Medical Research, New York.

* Paper read in abstract before the Society for Serology and Haematology, New York, April 7, 1916.

1. Morgenroth, J. and Levy, R.: Berl. klin. Wchnschr., 1911, **48,** 1560 and 1979. Morgenroth, J., and Kaufmann, N. M.: Centralbl. f. Bakteriol., 1912, **54,** Ref., Part 69; Ztschr. f. Immunitätsf., 1913, Orig., **18,** 145. Morgenroth, J., and Bumke, E.: Deutsch. med. Wchnschr., 1914, **40,** 538. Engewer, T.: Ztschr. f. Hyg. u. Infektionskr., 1913, **73,** 194. Gutmann, L.: Ztschr. f. Immunitätsf., 1912, Orig., **15,** 625. Levy, R.: Berl. klin. Wchnschr., 1912, **49,** 2486.

isopropylhydrocuprein, isobutylhydrocuprein and isoamylhydrocuprein. Indeed, if the value of ethylhydrocuprein in this respect be represented by 1, then that of isopropylhydrocuprein = ½ to ¼, isoamylhydrocuprein = 1/20 and quinin = 1/150. Isbutylhydrocuprein is no more potent in this respect than quinin itself, nor does hydroquinin appear to be of much greater value.

Shortly after the publication of these studies of Morgenroth and his collaborators, the drug ethylhydrocuprein was placed on the market under the trade name of "optochin" (the free base, the hydrochlorid, and, later, the salicylicacidester) and use was made of it in the treatment of acute lobar pneumonia in man. In the great majority of recorded cases it was administered by mouth.

Little being known regarding the dosage of this drug in man, it is not surprising that in several of the earlier cases its exhibition led to alarming and untoward symptoms, such as tinnitus, deafness, amblyopia or amaurosis, and that, even at the present time, opinion among clinicians is by no means unanimous regarding either its value or its dosage. The results of studies made by one of us (M.) on the action of ethylhydrocuprein on type strains of the four main serologic groups of pneumococci,[2] and on the pneumococcidal action acquired by the serum of rabbits after its administration,[3] led to the present investigation. Its main object was to discover how the dosage of the drug might be regulated in man. In this endeavor we were helped by a study of the literature, for the clinical experience therein recorded seemed to fix the upper limit of dosage, beyond which it was not safe to go, at about 1.5 gm. per twenty-four hours, given by mouth. In no case reported in the literature have we been able to discover the weights of the patients treated, and, accordingly, we have been forced to take this upper limit as that for a patient of average size.

It is difficult to arrive at a valuation of the drug in acute lobar pneumonia from a perusal of the published treated cases, because of contradictory opinions; whereas Wright[4] thinks it inefficacious or doubtfully efficacious, and Parkinson[5] states that it has little or no effect on the course of pneumonia in man, many authors express favorable opinions. It would appear that the earlier writers on this subject used unsuitable doses, while the later observers used a dosage more nearly approaching the optimum, and perhaps for this reason obtained better results.

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2. Moore, H. F.: Jour. Exper. Med., 1915, **22**, 269.
3. Moore, H. F.: Jour. Exper. Med., 1915, **22**, 551.
4. Wright, A. E., Morgan, W. P., Colebrook, L., and Dodgson, R. W.: Lancet, London, 1912, **2**, 1633 and 1701.
5. Parkinson, J.: Ztschr. f. Chemotherapie u. verw. Geb., 1913, Orig., **2**, 1.

We have collected from the literature 787 cases,[6] the details of which are more or less completely available. They are reproduced in tabular form in Table 1. Empyema in patients treated with the drug does not seem to have been a frequent complication, 1.9 per cent. The total mortality in the 787 cases recorded is 12.96 per cent., a figure in itself somewhat encouraging in the use of the drug.

SCOPE OF THE PROBLEM AND GENERAL CONSIDERATIONS

The present investigations had as its main object the solution of the following problems:

1. Is it possible to confer on the serum of patients of average size, by the administration of 1.5 gm. of ethylhydrocuprein per twenty-four hours, the power of killing pneumococci in the test tube?

2. If so, what is the optimum method of administration of the drug, both as regards the route, and the regulation of the size and spacing of the individual doses, as determined by a study of this acquired bactericidal property of the serum?

3. What is the effect of the drug on the course and mortality of acute lobar pneumonia in man, when the influence of the particular immunologic group of the infecting pneumococcus on the natural course of the disease is taken into consideration?

It is logical to suppose that if ethylhydrocuprein has any special value in the treatment of acute lobar pneumonia its efficacy must be due to its specific pneumococcidal action. Unless it can be given in such amounts that there is produced within the human organism a condition whereby pneumococci are destroyed in situ, or, at least, prevented from local or general migration, and from the establishment of their growth in previously uninfected regions, it seems unlikely that marked therapeutic results can be expected from its use.

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6. Baermann, G.: Ztschr. f. exper. Path. u. Therap., 1914, **15,** 476. Bieling: Therapie d. Gegenw., 1915, **56,** New Series **17,** 203. Dünner, L., and Eisner, G.: Therap. d. Gegenw., 1916, **57,** New Series **18,** 41. Von Dziembowski, S.: Deutsch. med. Wchnschr., 1915, **41,** 1571. Frank, E.: Berl. klin. Wchnschr., 1915, **52,** 421. Frank, G. V.: Zentralbl. f. inn. Med., 1916, **37,** 265. Fränkel, A.: Berl. klin. Wchnschr., 1912, **49,** 664; Therap. d. Gegenw., 1915, **56,** New Series **17,** 1. Hess, O.: München. med. Wchnschr., 1915, **62,** 1528. Kaufmann, M.: München. med. Wchnschr., 1915, **62,** 291. Lapinski, J.: Therap. Monatsh., 1915, **29,** 103. Lenné: Berl. klin. Wchnschr., 1913, **50,** 1984. Leschke, E.: Deutsch. med. Wchnschr., 1915, **41,** 1359. Loeb, A.: Berl. klin. Wchnschr., 1915, **52,** 1108. Loewe, E., and Meyer, F.: Berl. klin. Wchnschr., 1915, **52,** 1018. Manliu, J.: Berl. klin. Wchnschr., 1916, **53,** 58. Mendel: München. med. Wchnschr., 1915, **62,** 740. Moritz: Ztschr. f. ärtz. Fortbild., 1915, **12,** 321. Oliver, G. H.: Brit. Med. Jour., 1916, **1,** 580. Peiper, A.: Berl. klin. Wchnschr., 1915, **52,** 396. Rosenow, G.: Berl. klin. Wchnschr., 1915, **52,** 393. Rosenow, G.: Deutsch. med. Wchnschr., 1916, **41,** 791. Rosenthal, F.: Therap. d. Gegenw., 1915, **56,** New Series, **17,** 181. Schottmüller: Berl. klin. Wchnschr., 1915, **52,** 758. Silbergleit, H.: Berl. klin. Wchnschr., 1915, **52,** 1231. Simon, A.: Deutsch. med. Wchnschr., 1915, **41,** 643. Vetlesen: Berl. klin. Wchnschr., 1913, **50,** 1473.

TABLE 1.—Summary of Reported Cases of Acute Lobar Pneumonia Treated with Optochin[a]

Author	Number of Cases	Summary of Dosage per 24 Hours, in Gm.[a]	Disturbances of Vision	Disturbances of Hearing	Vomiting	Empyema	Mortality	Remarks
Baermann, G. ...	34	3 cases: 1 × 0.75; hydrochlorid intravenously	.....	.....	.....	.....	1	
		5 cases: 2-4 × 0.5, base (in oil) intramuscularly	.....	.....	.....	.....	2	
		11 cases: 2.2 × 0.5 or 4 × 0.25 base, (in oil) intramuscularly and 20-40 c.c. convalescent serum subcutaneously	.....	.....	.....	.....	2	One patient had miliary tuberculosis
		7 cases: 2-4 × 0.5; 6-8 × 0.25; hydrochlorid by mouth						
		8 cases: 2-4 × 0.5; 6 × 0.25; hydrochlorid by mouth and 20-50 c.c. serum subcutaneously	.....	.....	.....	1	1	
Bieling.....	16	3-4 × 0.5 hydrochlorid	.....	.....	.....	.....	2	Smaller doses in children
Dunner, L. and Eisner, G.	100	3 × 0.5; 6 × 0.25; hydrochlorid; when any toxic symptoms appeared the dose was diminished to 6-8 × 0.125-0.15, or drug was discontinued	(5 "glimmering" before eyes); 3 amblyopia; 1 amaurosis; all transient	9 tinnitus.....	4	2	12	One lung abscess
Frank, E.	3	4 × 0.5.....	1 amaurosis (improvement before death)	.....	.....	.....	1	
Frank, G. V.	40	3 × 0.5; 6 × 0.25; 4-5 × 0.25, base	1 amaurosis; 1 severe amblyopia (improvement before death); 3 slight amblyopia	1 deafness.....	.....	.....	10	
Fränkel, A. (I)....	21	2-5 × 0.5, hydrochlorid	3 amblyopia; optochin discontinued; transient	.....	1 or 2	.....	4 (severe cases)	
Fränkel, A. (II)...	13	3 × 0.5; hydrochlorid	.....	1 tinnitus.....	.....	.....	1	

Hess, O.	81	3 × 0.5; 6-8 × 0.2; 6 × 0.25; hydrochlorid	6 amblyopia, transient; 1 amaurosis; optochin stopped; recovery; 1 amaurosis; almost complete recovery	Occasional difficulty of hearing and tinnitus	.....	.....	10†	4-5 × 0.2 gm. for 1-2 days after temperature became normal
Kaufmann, M.	5	4 × 0.5; 6 × 0.25; hydrochlorid	1 amaurosis; transient.....					
	14	5 × 0.3; hydrochlorid	.....	Difficulty of hearing and tinnitus in several cases	Several cases	.....	2‡	Continues optochin 1-2 days after temp. becomes normal; continuous day and night dosage to prevent "fastness"
Lapinski, J.	35	3 × 0.5; 5-6 × 0.5; 8 × 0.5; 4 × 0.5; 12 × 0.5 (one case); hydrochlorid, base and salicylicacidester	1 slight amblyopia; 1 amaurosis; 1 amaurosis; all transient	.....	Occasional	1	5	
Lenné	17	1-4 × 0.4; 2-4 × 0.5; hydrochlorid	1 anaurosis, transient; (dilatation of pupils in several cases)	1 difficulty in hearing, transient	.....	1	2	Smaller doses to children under 15 years
	18	2-3 × 0.4-0.5, hydrochlorid, + serum (20-60 c.c.), 2 intravenous injections of 0.2 gm. each in one patient	.....	.....	1	.....	3	
Leschke, A.	3	10 × 0.2; optochin salicylicacidester; when temp. drops 5 × 0.2						
Loeb, A.	24	6 × 0.25; hydrochlorid and salicylic acid ester	1 amblyopia, transient; (several slight disturbances; transient)	Several times tinnitus				
Loewe E., and Meyer, F.	43	4-6 × 0.25; hydrochlorid	.....	1 tinnitus and difficulty of hearing, transient	1	1	2	
Manlieu, J.	12	6 × 0.25; hydrochlorid or base	2 slight amblyopia; transient. (1 widening of pupils; optochin was discontinued)	1 tinnitus.....	.....	.....	2§	Milk diet with base
Mendel	12	5 × 0.3, base	1 amblyopia; transient.....	Occasional tinnitus	.....	.....	.....	Milk diet
Moritz	28	3 × 0.5						
Oliver, G. H.	1	8 × 0.3 (total 120 grains; 7.7 gm.)	Amaurosis; vision improved, but later declined; vision still much impaired 1 year after the drug was given	Tinnitus and partial deafness during administration				

* By mouth unless otherwise stated.
† Two mixed infection with streptococci.
‡ One mixed infection with streptococci; one Grave's disease.
§ One nephritis; one pericarditis.

TABLE 1.—Summary of Reported Cases of Acute Lobar Pneumonia Treated with Optochin—(*Continued*)

Author	Number of Cases	Summary of Dosage per 24 Hours, in Gm.*	Disturbances of Vision	Disturbances of Hearing	Vomiting	Empyema	Mortality	Remarks
Parkinson, J.	9	1-4 × 0.5; hydrochlorid by mouth; 1 × 0.125—3 × 0.5; hydrochlorid subcutaneously	(3 dilatation of pupils)			2	2	
Peiper, A.	31	2 × 0.25; 2-3 × 0.5; 6 × 0.25	1 slight amblyopia, transient; 1 amaurosis, transient				6	
Rosenow, G. (I)	26	6 × 0.25; hydrochlorid and salicylicacidester				1	2	
Rosenow, G. (II)	34	6 × 0.25 hydrochlorid by mouth; 3 × 0.2 hydrochlorid by mouth; 6-8 × 0.25 salicylicacidester by mouth; 6-8 × 0.25 base, by mouth; 1 × 0.5 hydrochlorid intraven.		1 tinnitus, 1 difficulty in hearing		4	2	Continues drug for a few days after temperature drops
Rosenthal, F.	6	3 × 0.5; 6 × 0.25; hydrochlorid					1¶	
Silbergleit, H.	40	6 × 0.25; 6 × 0.2; hydrochlorid	1 slight amblyopia	Tinnitus		1	5	
Simon	57	3-6 × 0.25; ester and hydrochlorid (exceptionally 3 × 0.5)	2 amblyopia, transient	12 difficulty in hearing, transient			15	Better results in early cases
Schottmüller	3	1.5 gm. per 24 hours					1	
Vetlesen	9	3 × 0.5 hydrochlorid		3 tinnitus and deafness, transient				
Warburg	41	3 × 0.5; 6 × 0.25; hydrochlorid base, salicylicacidester		Tinnitus and difficulty in hearing in a few cases		1	6	
Totals	787		36 (4.57 per cent.) amaurosis or amblyopia			15 (1.9%)	102 (12.96%)	

¶ Typhoid, convalescent.

Wright[4] was the first to show that the serum of human patients receiving the drug by mouth destroyed pneumococci in the test tube. It has been shown by one of us (M.)[5] that the serum of normal rabbits which had received a single comparatively large but well tolerated dose of ethylhydrocuprein hydrochlorid acquires an inhibitory and bactericidal action on pneumococci in the test tube; furthermore, that this bactericidal action resulting from a single dose lasts but a couple of hours in the living animal, and then disappears. When rabbit serum possessing this property is inoculated with a small amount of an eighteen-hour broth culture of pneumococcus and incubated at 37 C., the destruction of any considerable number of pneumococci requires a period of several, often as many as forty, hours, the time required depending in part on the degree of bactericidal power which the serum possesses and in part on the number of pneumococci present. It follows, therefore, that, in order to destroy pneumococci within the living animal, the drug should be repeatedly administered in suitable doses, at comparatively short intervals of time, in order that the pneumococcidal action in the circulating fluids may be continuously preserved for the necessary length of time.

Any effort to cure a pneumococcal septicemia or pneumonia through the administration of ethylhydrocuprein must aim at (1) the production of a pneumococcidal action in the blood serum; (2) its maintenance as constantly as possible over a period of time sufficiently long to insure the destruction of all the infecting pneumococci; and, (3), the production of a concentration of the drug within the body which is harmless to any part thereof.

Marked success has not attended our efforts to cure pneumococcal septicemia in the rabbit, owing to the susceptibility of these animals to such amounts of the drug as are necessary for the production of a constant bactericidal action in the blood stream for a sufficiently long period.

In acute lobar pneumonia it should not be surprising if the time required for such an agent as optochin to destroy all the pneumococci in the lung lesion were greater than that necessary to destroy those circulating in the blood stream, because it is possible that the majority of pneumococci in the lung lesion may be mechanically protected by the exudate from the action of the drug. Indeed, this would seem to be, in part, the explanation of the fact, pointed out by Morgenroth,[7] Leschke[8] and others, that the results of optochin treatment in lobar pneumonia are better the earlier after the onset of the disease it is instituted; in other words, before any considerable consolidation of

7. Morgenroth, J.: Berl. klin. Wchnschr., 1914, **51,** 1829 and 1865.
8. Leschke, E.: Deutsch. med. Wchnschr., 1915, **41,** 1359.

the affected lung has taken place. On the other hand, we might expect that a much shorter time would be required for the destruction of pneumococci circulating in the blood stream.

There is still another consideration of importance in the treatment of lobar pneumonia with optochin, namely, the possibility that a dosage which is too small, apart from failing to confer a pneumococcidal action on the circulating blood, may give rise to conditions which favor the acquisition by the infecting pneumococci of a condition of resistance or "fastness" to the drug, so that even a later increase in dosage may be insufficient to destroy the bacteria and hence be of no avail.

Morgenroth and Kaufmann[9] have shown that, in mice infected with pneumococci and insufficiently treated with ethylhydrocuprein, the pneumococci are able to react against the drug so that, after four passages through such insufficiently treated mice, the strain may become completely resistant, or "fast," to its action. A similar phenomenon can be observed in the test tube. Tugendreich and Russo[10] have shown that, by subjecting pneumococci for two hours daily, at room temperature, to gradually increasing and at first sublethal concentrations of optochin and incubating in serum-broth in the intervals, the micro-organisms could be educated, so to speak, in ten or twelve days, to grow in comparatively high concentrations of the drug. We, ourselves, carrying out similar observations at 37 C., have seen, in six subcultures made in gradually increasing concentrations of optochin, a strain of pneumococcus become capable of growing in a concentration of the drug greater than that previously sufficient to kill it.

We feel that these phenomena are of significance in the present problem, namely, the chemotherapy of pneumonia with ethylhydrocuprein, since it is possible that here the conditions may at times favor the micro-organism, in that the pneumonic exudate may conceivably afford some degree of protection to the pneumococci from the circulating chemotherapeutic agent. Assuming that this may be so, it is possible, in the treatment of pneumonia with optochin, that the pneumococci may be hindered from reacting against and becoming fast to the drug, by giving to the patient such doses of optochin as will, within limits of safety, confer on the circulating blood the maximum bactericidal action in the shortest space of time, and by maintaining this action as nearly as possible at a constant level throughout the treatment. In other words, the development on the part of the parasite of a condition of fastness is to be regarded as a vital action, and one object of the therapy is to subject the infecting pneumococci to such conditions as

9. Morgenroth, J., and Kaufmann, M.: Ztschr. f. Immunitätsf., 1912, Orig., **15**, 610.

10. Tugendreich, J., and Russo, C.: Ztschr. f. Immunitätsf., 1913, Orig., **19**, 156.

will prevent them from exercising any vital activity. Fortunately, such an adaptation of the parasite to these influences seems to require a considerable space of time for its development; apparently, several days of exposure to nonfatal concentrations of optochin are necessary before the pneumococcus acquires such a degree of "fastness" that it can carry on its activities in the presence of the degree of bactericidal action that may readily be produced in the circulating fluids by the administration of optochin. In the actual treatment of disease, therefore, with such a chemotherapeutic agent as optochin, it would seem desirable to avoid such a low concentration of the drug in the blood as would permit the pneumococci to develop the property of "fastness." A case bearing on this point will be cited later.

Bearing in mind the foregoing considerations, one is led to believe that the ideal to be attained in the use of ethylhydrocuprein in acute lobar pneumonia is to regulate the administration of the drug so that,

(1) within the limit of safety, a maximum bactericidal action may appear in the circulating blood within the shortest possible time after the commencement of the specific treatment; and,

(2) the bactericidal action may be maintained as constantly as possible, as long as the administration of the drug is required.

The first object, namely, the rapid production of the bactericidal action in the blood stream, might be attained by an intravenous injection. This, however, did not commend itself to us, because the work of one of us (M.),[8] on intravenous injection of the drug in rabbits, has shown that toxic signs may result from doses so small that but little bactericidal action results in the serum. Boecker,[11] also, after intravenous administration, was unable to demonstrate considerable bactericidal action in the serum of rabbits, and was only slightly more successful with guinea-pigs. Further, a case was reported by Smith and Fantus[12] in which intravenous injections of optochin resulted in numbness of the lower extremities, impaired vision and deafness, and in which death was attributed by these authors to cardiac depression, which was considered to be in part due to the intravenous administration of the drug. Baermann,[13] however, has given intravenous injections of single doses of 0.75 gm. of the drug without recording ill effects. Rosenow[14] also used intravenous injection of 0.5 gm. Likewise, subcutaneous injection did not seem to us desirable, in general, in view of the painful infiltration to which it may give rise.[8] As will be mentioned below, intramuscular administration has also been tried in the present study, with unsatisfactory results. Consequently, oral

11. Boecker, E.: Ztschr. f. Immunitätsf., 1915, Orig., **24,** 148.
12. Smith, M. I., and Fantus, B.: Jour. Pharmacol. and Exper. Therap., 1916, **8,** 53.
13. Baermann, G.: Ztschr. f. exper. Path. u. Therap., 1914, **15,** 476.
14. Rosenow, G.: Deutsch. med. Wchnschr., 1915, **41,** 791.

administration has been adopted as a general method; the absorption of the hydrochlorid of the drug from the alimentary canal seems to be so rapid that the requirement under discussion, namely, a rapid appearance of the bactericidal action in the serum, is capable of fulfilment by using this route.

The proposed ideal of constant maintenance of the bactericidal action required study of the regulation of the size and spacing of the individual doses designed to produce this result.

If 1.5 gm. for an individual of average size be decided on as the total amount of the drug to be given per twenty-four hours, this quantity may be subdivided in several ways. The method of administration most likely to give rise to a fulfilment of the condition outlined above is that of giving one or several initial relatively large doses of the drug (for example, 0.45 to 0.5 gm.), followed by several smaller ones at regular intervals. It is less likely that several small doses of, say 0.15 gm., would produce a bactericidal action within as short a space of time as the former method. But, whatever method of regulating the doses be employed, if the rates of absorption and elimination or destruction of the drug be comparable in different individuals, then the body weight, through its influence on blood volume, should play an important part in the resulting concentration of the drug in the blood stream; in other words, the concentration of optochin should be greater in those patients who receive a greater amount per kilogram of body weight. If these views are correct, a certain minimum amount of optochin must be absorbed per kilo of body weight within a definite time in order that any bactericidal action in the blood stream may result. Evidence in favor of such a view will be brought forward later. It will suffice now to suggest that, in order to produce in the blood stream the desired bactericidal action, the possibility of a relationship between the body weight of the patient and the amount of optochin given per twenty-four hours must be taken into consideration.

CHARACTER OF THE CASES AND THEIR GENERAL TREATMENT

The present study comprises observations on thirty-two patients suffering from acute lobar pneumonia, admitted to the wards of the Hospital of The Rockefeller Institute during the months November, 1915, to July, 1916. Each of these thirty-two patients received optochin[15] hydrochlorid, and in twenty-eight of them a detailed study

15. The optochin used in this study was obtained through Merck & Co., New York, was manufactured by Vereinigte Chininfabriken, Zimmer & Co., Frankfort a. M., and the bottles containing it bore the number 11,695. It was a white powder. We have tested experimentally other specimens of optochin which were more or less yellowish-white in color, but they apparently were not as potent as the drug used in the present study and one of them was found to contain impurities. It is important that the drug should be free from impurities if a uniform system of dosage is to be employed.

of the blood serum was made with reference to the presence or absence of bactericidal power for the pneumococcus. With one exception, a strain of pneumococcus was isolated from every case, and differentiated according to the biologic classification proposed by Dochez and Gillespie[16] and later Avery.[17] The cases treated were due to infection with representatives of all the main groups of pneumococci, although the majority were infected with strains belonging to Group II and Group III (*Pneumococcus mucosus*). Only two cases of infection with pneumococci belonging to Group I were treated with optochin, and of these, one received, in addition, several doses of antipneumococcus serum (Group I) intravenously; all other cases of infection with strains of this group were purposely not treated, owing to the employment of other therapeutic methods. Five cases of infection with strains of Group II were treated, in addition to the optochin, with "extract" of the type homologous antipneumococcus serum,[18] which was prepared according to Chickering's[19] modification of the method described by Gay[20] and the former investigator. Two cases due to infection with *Pneumococcus mucosus* (Group III) were likewise treated with the extract of the corresponding antiserum[21] as well as with optochin. No attempt was made to select the patients for treatment, so that, for purposes of comparison, this series may be considered to be a fairly representative one. The extremes of age were 15 and 66 years, the average age 37.9 years. The incidence of the cases according to decade is given in the following tabulation:

Decade	No. of Cases
11 to 20 inclusive	5
21 to 30 inclusive	7
31 to 40 inclusive	7
41 to 50 inclusive	5
51 to 60 inclusive	5
61 to 70 inclusive	3

Apart from the optochin or serum therapy, the general treatment of the patients was that usually carried out in this hospital. Digipuratum was used when indicated, and it may be stated that this drug has no effect on the bactericidal action in the serum resulting from optochin therapy, nor does it confer such a property itself. The use of camphor was avoided except in two cases towards the end of the

16. Dochez, A. R., and Gillespie, L. J.: Jour. Am. Med. Assn., 1913, **61**, 727.
17. Avery, O. T.: Jour. Exper. Med., 1915, **22**, 804.
18. Such extract has no bactericidal action on the pneumococcus in the test tube.
19. Chickering, H. T.: Jour. Exper. Med., 1915, **22**, 248.
20. Gay, F. P., and Chickering, H. T.: Jour. Exper. Med., 1915, **21**, 389.
21. The particular serum from which this extract was obtained was kindly supplied by Dr. Wadsworth of the New York State Board of Health.

treatment because Rosenthal[22] has shown that its administration diminishes the curative effect of optochin in animals infected with pneumococci. It does not appear that any particular diet has an important effect on the bactericidal action resulting in the serum of patients treated with optochin hydrochlorid.

In the present study the free base of ethylhydrocuprein or the salicylicacidester has not been used; the hydrochlorid alone has been employed.

TECHNIC EMPLOYED; INTERPRETATION OF RESULTS

The bactericidal action of the serum was studied as follows: Specimens of blood were obtained from the veins at the elbow, before and at frequent intervals during the administration of the drug. The blood was allowed to clot and to stand in the refrigerator over night; the serum was then separated and kept in the refrigerator until all specimens had been assembled, when the tests were carried out.[23] In two cases in which the patients had positive blood cultures, the serum was heated at 56 C. for thirty minutes in order to kill any living pneumococci that might be present; except for these instances the serum was not inactivated, since the bactericidal power of the serum of patients who have received optochin is not appreciably increased or diminished by the process of inactivation. Serum was used for the tests after it had been ascertained that the whole blood was no more bactericidal than the former. Where one specimen of blood was obtained in the interval between two doses of optochin, as a general rule the bleeding immediately preceded the administration of the second dose.

Virulent laboratory strains of pneumococci, long accustomed to growth outside the body, were used for the tests. Such strains are more resistant to optochin than strains freshly isolated from the human body. In the majority of instances the particular strain used belonged to an immunologic group other than that causing the infection. In cases in which the infecting microorganism belonged to the same group as that of the strain used for the bactericidal tests, agglutination tests (microscopic and macroscopic) were carried out with the patient's serum, in order to rule out the possibility that the patient had acquired agglutinins within his serum, the presence of which might give rise to a simulation of bactericidal action, thus introducing an obvious fallacy.

Bactericidal substances for the pneumococcus are not found among the antibodies demonstrable about the time of the crisis in patients with acute lobar pneumonia, nor is an artificially prepared immune serum itself pneumococcidal; hence, any bactericidal action observed in the serum must have been due to the optochin treatment.

Most of the tests were carried out with cultures from four to six hours old. The age of the culture selected for carrying out bactericidal tests is by no means an unimportant detail. In a previous communication one of us (C.)[24] has reported the results of an investigation of the nature of bacterial lag, as it occurs in bouillon cultures of pneumo-

22. Rosenthal: Berl. klin. Wchnschr., 1915, **52**, 709.

23. The serum retains, undiminished, the bactericidal action resulting from the administration of optochin for a considerable period of time when kept in the refrigerator.

24. Chesney, A. M.: Jour. Exper. Med., 1916, **24**, 387.

coccus; in that work it was shown that actively growing (four to eight hours at 37 C.) cultures of pneumococcus, on transplantation into new mediums, exhibit no delay in growth but continue to grow rapidly; whereas, cultures twelve hours and older, on transplantation, require several hours (one to four or more) before rapid growth begins to take place. Furthermore, evidence was therein presented which indicates that this initial inhibition of growth, or "lag," exhibited by older cultures of pneumococci, is due to an injury caused by the action of the metabolic products of the bacteria themselves on that structure or function of the bacteria which has to do with the growth of the micro-organisms. On the bases of the facts brought out in that investigation, it was suggested that, in determining the bactericidal power of any agent, it might be desirable to carry out the tests with "young" cultures; that is to say, with such cultures as had not sustained an injury to their powers of growth and would, therefore, on transplantation, exhibit no lag. Otherwise, if cultures, the individuals of which had already been partially injured, were subjected to the action of a bactericidal agent, one would have to deal with a summation of effects, which, in turn, might give rise to an exaggerated idea of the efficacy of the agent tested. Support for this view is offered by the work of Chick[25] who has presented evidence which indicates that the individual organisms of a three-hour culture of *B. paratyphosus* are more resistant to the action of disinfectants than are those of a twenty-four-hour culture of the same strain. We shall present evidence later to show that the organisms of a four-hour culture of pneumococcus are more resistant to a bactericidal agent (optochin) than those of a twenty-hour culture of the same strain.

Moreover, the use of an actively growing culture affords the opportunity of measuring more accurately the degree of inhibitory or bactericidal power of the specimens tested, and thus, possibly, of estimating the relative amounts of the bactericidal agent present.

In actual practice, in the present study, it was found best to use a broth culture of pneumococcus which had been incubated at 37 C. for from four to six hours, so that it was just beginning to show a faint turbidity. Usually 0.001 c.c. of this culture was inoculated into each of the tubes containing 3.0 c.c. of the various specimens of serum to be tested. After all the tubes had been inoculated, 0.5 c.c. was removed from each, mixed with 20 c.c. of melted 1 per cent. glucose agar which had previously been cooled to 40 C., and plated at once. This amount of agar was sufficient to dilute the bactericidal agent in the serum to such an extent as to allow the pneumococci to grow on the plates. The tubes were then incubated in the dark in the water bath at 37 C. and, at suitable intervals thereafter, an equal amount (0.5 c.c.) was again removed from each tube and plated. All plates were incubated forty-eight hours and then the number of colonies on each was counted. It should be noted that "young" (four- to eight-hour) cultures of pneumococci contain many short

25. Chick, H.: Jour. of Hyg., 1908, **8**, 92.

chain forms and that each chain would give rise to only one colony on the plate. Nevertheless, since the specimens of serum of each case are tested with the same culture, the results in any one case are comparable; and, where the cultures used in testing the serums of different patients are of the same age, and grow at approximately the same rate, the results are comparable in different cases.

INTERPRETATION OF RESULTS

When actively growing pneumococci are subjected for twenty-four hours at 37 C. to the action of the serum of patients who have received optochin, several phenomena may take place. The micro-organisms may show, (1) unrestricted growth; (2) an initial temporary inhibition followed subsequently by unrestricted growth; (3) complete inhibition with little or no destruction of bacteria; or, finally, (4) there may be actual bactericidal action. The three phenomena last mentioned shall be hereafter designated "temporary inhibition," "complete inhibition," and "bactericidal action," respectively. They represent variations in degree of the same general process, namely, toxic action of optochin on the pneumococcus. Before attributing significance to the variations in pneumococcidal or inhibitory power, it is essential to determine whether or not they represent differences in the amount of optochin present in the serum. In order to ascertain this point, bactericidal tests were conducted with known concentrations of optochin in normal horse serum and "young" (four hours at 37 C.) and "old" (twenty hours at 37 C.) bouillon cultures of pneumococcus. The details of one of these experiments follows.

Experiment 1.—Varying dilutions of optochin in normal horse serum were made, ranging from 1:50,000 to 1:5,000,000. Into separate tubes containing 6 c.c. each of these dilutions there was inoculated 0.002 c.c. of a four-hour (37 C.) broth culture of pneumococcus, Group II. After thorough mixing, 0.5 c.c. was removed from each tube separately and plated in 1 per cent. glucose agar. The tubes were then incubated in the water bath at 37 C. and at suitable intervals 0.5 c.c. was removed from each tube and plated separately. All plates were incubated for forty-eight hours before counting. The same procedure was carried out with the same culture of pneumococcus when it was twenty hours old (37 C.), in order to contrast the behavior of the two under the same conditions (inoculation 0.0001 c.c.). The growth of the two cultures in the varying dilutions of optochin in normal horse serum is shown in Table 2.

Table 2 shows that dilutions of optochin in normal horse serum, varying in strength from 1:5,000,000 to 1:2,000,000, inclusive, exerted no apparent effect on either a four-hour or a twenty-hour culture of pneumococcus. In a dilution of 1:1,000,000 slight temporary inhibition was observed in the case of the four-hour cultures, whereas the twenty-hour culture showed a slight prolongation of the "lag" exhibited by this culture in control serum without optochin. In the next lower dilution, 1:800,000, the four-hour culture showed an unmistakable "lag" or temporary inhibition and the twenty-hour culture showed a decidedly lengthened "lag." In dilution of 1:600,000 there was almost

TABLE 2.—Bactericidal Action of Various Dilutions of Optochin in Normal Horse Serum

Dilution of Optochin in Normal Horse Serum	Four-Hour Culture Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*					Twenty-Hour Culture Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated†				
	Immediately	After 1½ Hrs. Inc.	After 4 Hrs. Inc.	After 6½ Hrs. Inc.	After 21 Hrs. Inc.	Immediately	After 1½ Hrs. Inc.	After 3½ Hrs. Inc.	After 6½ Hrs. Inc.	After 21 Hrs. Inc.
Control serum (no optochin)	321	752	A. C.‡	C.	C.	255	188	184	20,000 Est.	C.
1:5,000,000	300	760	A. C.	C.	C.	281	172	139	20,000 Est.	C.
1:3,000,000	308	770	A. C.	C.	C.	257	162	158	20,000 Est.	C.
1:2,000,000	286	758	A. C.	C.	C.	822	183	150	20,000 Est.	C.
1:1,000,000	291	705	20,000 Est.	C.	C.	284	205	191	15,000 Est.	C.
1:800,000	292	352	1,230	20,000 Est.	C.	200	225	149	164	C.
1:600,000	297	340	383	354	745	305	170	75	87	0
1:400,000	306	301	219	43	0	304	139	49	20	0
1:300,000	296	270	186	30	0	305	78	42	5	0
1:100,000	308	337	93	12	0	248	49	11	1	0
1:50,000	355	207	31	4	0	186	18	9	0	0

* This culture was not multiplying as rapidly as is usually the case in "young," four-hour cultures. Inoculation: 0.001 c.c. of broth culture of pneumococcus, Group II.

† Inoculation: 0.0001 c.c. of broth culture of pneumococcus, Group II.

‡ In this and the following table A. C. = almost confluent; C = confluent; Est. = estimated.

complete inhibition in the case of the four-hour culture and bactericidal action in that of the twenty-hour culture. In the lower dilutions there was manifested a progressive increase in the bactericidal action of the optochin-containing serum. The experiment shows that variations in the concentration of optochin in normal horse serum are accompanied by corresponding differences in the degree of specific action of the drug on the pneumococcus, and that this method is a reliable one for detecting slight differences in the amount of the drug present in the serum.

The experiment further demonstrates that the individual members of a four-hour broth culture of pneumococcus are more resistant to the action of optochin in horse serum than are the members of the same culture when it has been incubated for twenty hours. This is clear from the more rapid rate at which the pneumococci of the twenty-hour culture are destroyed than that at which the organisms of the four-hour culture succumb, and, in addition, by the observation that optochin in a dilution of 1:600,000 sufficed to exert a bactericidal action on the

older culture, while on the younger it was able to exert an inhibitory effect only. The experiment, therefore, may be said to have borne out the conceptions that were formed as a result of the investigation of the nature of bacterial lag[24] in regard to differences in the resistance of members of a culture of bacteria depending on the age of the culture itself. In such tests as have been carried out in this investigation "young," actively-growing cultures commend themselves for use, since, by their employment, the phenomenon of "lag" is avoided in the controls and the interpretation of results is not confused by its presence.

Not infrequently, when such "young" cultures are used in bactericidal tests, the plates poured after incubation of the serum for one or two hours may show a larger number of colonies than those poured immediately after inoculation; whereas, the plates poured after further incubation (five to twenty hours) of the same specimen of serum, may show a decided decrease in the number of colonies, or even sterility. This temporary initial increase in the number of viable pneumococci may be due to the breaking up of chain forms, which are numerous in "young" broth cultures of pneumococcus; or, it may be due to an appreciable interval of time being required for the bactericidal agent to exert its full effect on the organisms, during which interval some new individuals are formed.

The behavior of the pneumococci in the various dilutions of optochin in normal horse serum and in the serum of patients receiving the drug, has also been followed from the microscopic standpoint. Smears were made at frequent intervals during incubation and stained to show the capsules with Hiss' capsule stain. It was found that pneumococci, after some hours exposure to nonlethal concentrations of optochin in serum, possessed a much larger capsule with a denser outer zone than did the pneumococci grown in serum without optochin. Furthermore, after several hours' exposure the capsules were found to be progressively larger the greater the concentration of optochin to which the organisms had been exposed, provided the concentration was not great enough to exert bactericidal action; frequently, in still greater concentrations, there were vacuoles in the capsular substance, giving it a moth-eaten appearance. The meaning of these phenomena is not clear.

DOSAGE OF OPTOCHIN

The patients treated fall into two groups, depending on the route of administration, as follows:

I. Those receiving all doses by mouth.

II. Those receiving one or several doses intramuscularly at the outset, and all subsequent doses by mouth.

I. ORAL ADMINISTRATION ENTIRELY

Twenty-eight patients were treated in this manner. In general, three systems of dosage were employed, which, for the sake of brevity, will be designated A, B and C.

THE VARIOUS METHODS OF DOSAGE USED; COMPARISON

Dosage A consisted of three doses of 0.5 gm. or 0.45 gm. each, given at regular intervals in the first twenty-four hours, followed in the second twenty-four hours by one dose of 0.45 gm. and seven of 0.15 gm. at regular intervals; thereafter nine or ten doses of 0.15 gm. in every twenty-four hours.

Dosage B consisted of ten doses of 0.15 gm. each, given at regular intervals in every twenty-four hours.

Dosage C consisted, in patients of average size, of a single initial dose of 0.45 gm., followed at regular intervals by single doses of 0.15 gm. each, the total amount of optochin given in twenty-four hours being the same for all periods of twenty-four hours, namely, 1.5 gm. Where patients were below average size and received less than 1.5 gm. in twenty-four hours, the same scheme of separation of doses was adhered to, and their size was proportionately decreased.

In contrasting the results obtained from administering the drug to different patients according to these various methods of dosage, care has been taken to select from the cases studied only those which are comparable as regards (1) amount of drug given per kilogram of body weight per twenty-four hours, and (2) details of bactericidal tests, such as use of young cultures and of inoculations of comparable numbers of pneumococci. Only cases which fulfil these conditions have been selected for the comparison of the various methods of dosage.

Dosage A.—Several relatively large doses at the outset, followed by numerous smaller ones.

Two cases in which the patients were treated according to this method will be presented for analysis. Details of the cases follow, together with tables showing the results of a study of the bactericidal power of the serum of each case. In addition, opportunity will be afforded in the first case (Case 1, Hosp. No. 2600) to contrast the inhibitory and bactericidal effect of the serum on "young" (four-hour) actively growing cultures of pneumococci with that on "old" (twenty-hour) cultures of the same strain.

CASE 1 (No. 2600).—C. C., man, waiter, aged 34, weight 65.4 kg.

Past History.—Unimportant.

Present Illness.—Chill, pain in chest, cough, and bloody sputum three days before admission.

Status on Admission: temperature, 102.2 F.; pulse, 98; respirations, 40;

beginning involvement of right lower lobe; white blood count, 15,000; blood culture sterile; sputum mucopurulent, streaked with blood and containing pneumococcus, Group IV.

Course: On the day following admission the patient was more seriously ill. The temperature rose to 105 F.; the physical signs indicated frank consolidation of the right lower lobe, and blood culture taken at this time showed the presence of pneumococcus, Group IV. Optochin was started on this, the fifth, day of the disease. During the first twenty-four hours three doses of 0.5 gm. each at intervals of eight hours were given; during the second twenty-four hours 0.45 gm. was given in one dose, followed after another interval of eight hours by seven doses of 0.15 gm. each at intervals of two hours; thereafter the drug was given at the rate of ten doses of 0.15 gm. each in twenty-four hours. The patient's condition improved after the optochin was begun, his temperature (rectal) reaching 100 F. on the seventh day, forty-eight hours after optochin was started, and normal on the eighth day. The affected lobe cleared up slowly, although the temperature remained normal. Blood culture, which was positive before treatment was begun, was sterile twenty-four hours after the optochin was started. There was no spread of the pneumonic process to other lobes. Total amount of optochin given, 3.6 gm. No toxic symptoms referable to the optochin were observed. Amount of optochin given per kilo of body weight per twenty-four hours, 0.0229 gm.

TABLE 3.—Bactericidal Test of Serum of Case 1 (No. 2600), Showing Differences Obtained with "Young" and "Old" Cultures

Time in Hours After Initial Dose of Optochin	Doses of Optochin in Gm. Following Corresponding Test Bleeding	Specimen of Serum Tested	Four-Hour Culture. Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*				Twenty-Hour Culture. No. of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated†		
			Immediately	After 1½ Hrs. Inc.	After 4½ Hrs. Inc.	After 22 Hrs. Inc.	Immediately	After 5 Hrs. Inc.	After 26 Hrs. Inc.
0.0	1 × 0.5	1	951	8,000	A. C.	C.	210	A. C.	C.
3.0	.....	2	867	1,128	20,000 Est.	C.	179	229	50
6.0	.....	3	934	960	10,000	A. C.	277	74	
8.0	1 × 0.5	4	908	2,250	20,000 Est.	C.	190	240	23
11.0	.....	5	876	997	1,220	308	224	4	0
16.0	1 × 0.5	6	970	1,065	2,200	2,000	131	9	0
19.0	.....	7	908	905	745	21	227	2	0
22.0	.....	8	900	709	1,155	180	256	1	0
24.0	1 × 0.45	9	892	888	1,228	16	275	1	0
26.5	.....	10	973	842	1,370	2	53	0	0
29.5	.....	11	980	845	1,565	84	127	0	0
31.75	1 × 0.15	12	885	960	1,271	13	217	0	
34.0	1 × 0.15	13	810	972	1,052	8	176	1	
36.0	1 × 0.15	14	743	903	1,447	23	75	0	0
38.0	3 × 0.15	15	857	970	476	1,500			
40.5	1 × 0.15	16	868	903	1,169	22	31	0	0
43.0	4 × 0.15	17	891	816	1,020	60	67	0	0

* Inoculation: 0.001 c.c. of broth culture of pneumococcus, Group II.
† Inoculation: 0.00001 c.c. of broth culture of pneumococcus, Group II.

Table 3 shows the difference in the results obtained, depending on whether "young" or "old" cultures are used in such tests. In the case of the twenty-hour-old culture bactericidal action appeared in the second specimen of serum, obtained three hours after the initial dose of optochin; whereas, in the case of the four-hour-old culture bactericidal action was not apparent until several hours later, namely, at eleven hours after the initial dose (Specimen 5). The results of this experiment confirm those obtained in Experiment 1, in which the action of known strengths of optochin in normal horse serum was tested on the same bouillon culture of pneumococcus after four hours' incubation of the same at 37 C., and after twenty hours' incubation, and in which the differences in resistance of the two cultures were demonstrated. This experiment further confirms the preceding one, in that it shows that by using a "young" (four hours at 37 C.) culture in carrying out such tests, it is possible to bring out finer differences in the degree of inhibitory power of the serum tested than is the case if an "old" (twenty hours at 37 C.) culture be employed. For example, this patient (who received 0.0229 gm. of optochin per kilogram of body weight per twenty-four hours), according to the tests with the young culture, acquired a bactericidal action in his serum between eight and eleven hours after the initial dose. Previous to this time temporary inhibition only was present, and close scrutiny of the figures will show that the degree of this inhibitory action varied in the different specimens. Thus, the third specimen of serum, obtained six hours after the initial dose of optochin, showed a greater degree of inhibitory action than did the next specimen obtained two hours later and immediately before the patient received the next dose of optochin. These facts would suggest that at the end of eight hours after the initial dose of 0.5 gm., the blood was already beginning to lose some of the optochin that had been absorbed earlier. Again, the fifth specimen of serum, obtained three hours after the second dose of optochin, showed bactericidal action, whereas the next specimen of serum, obtained eight hours after the second dose and immediately before the third dose, exhibited almost complete inhibition, but no bactericidal action, indicating again variations in bactericidal power within eight hours. On the other hand, with the twenty-hour culture no such clear-cut fluctuations in bactericidal action are observed. It is thus possible, with the aid of young, actively growing cultures, to detect in the serum of patients variations in the degree of specific inhibitory power, and therefore in the concentration of optochin, which would probably not be detected at all, or at least would not be as clearly demonstrated, if twenty-hour cultures were employed instead.

Case 2 (No. 2705).—B. B., housewife, aged 34, weight 52.8 kg.

Past History.—Unimportant.

Present Illness.—Chill, pain in the chest and cough twenty-four hours before admission to the hospital; blood-tinged sputum day of admission.

Status on Admission: temperature, 104.3 F.; pulse, 100; respirations, 34; cyanosis; dulness and suppression of breath sounds over the right lower lobe. Roentgenogram shows shadow in this area. White blood count, 40,000; blood culture sterile; sputum tenacious, hemorrhagic, containing *Pneumococcus mucosus.*

Course: Optochin administration was begun about twenty-four hours after the initial chill. The patient received in the first twenty-four hours three doses of 0.45 gm. each at intervals of eight hours. In the second twenty-four hours she received one dose of 0.45 gm. followed by seven doses of 0.15 gm. each, and in the two succeeding periods of twenty-four hours, nine doses of 0.15 gm. each. In the remaining periods of twenty-four hours, ten doses of 0.15 gm. each were given. The signs of consolidation gradually became more marked over the right lower lobe, and on the sixth day of the disease, five days after commencement of optochin, the physical signs indicated involvement of the left lower lobe, which was borne out by the roentgenogram. At this time a series of intravenous injections of extract of antipneumococcus serum (Group III) was begun, in addition to the optochin. In spite of treatment, the patient from this time on

TABLE 4.—Bactericidal Test of Serum of Case 2 (No. 2705)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Gm. Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 6 Hours Ina.	After 22½ Hours Inc.
0.0	1 × 0.45	1	1,090	C.	C.
3.0	.....	2	1,024	900	5,000
6.0	.....	3	1,005	934	C.
8.0	1 × 0.45	4	1,100	4,650	C.
11.0	.....	5	1,000	454	2
14.0	.....	6	1,000	764	35
16.0	1 × 0.45	7	1,080	779	968
19.0	.....	8	1,016	806	1
22.0	.....	9	1,050	519	784
24.0	1 × 0.45	10	1,000	631	10
26.0	.....	11	1,028	209	0
32.0	2 × 0.15	12	1,080	366	0
37.0	2 × 0.15	13	1,032	257	1
42.0	2 × 0.15	14	980	345	0
47.0	4 × 0.15	15	1,070	236	0
58.5	3 × 0.15	16	950	98	0
66.5	7 × 0.15	17	1,048	.....	0
85.5	9 × 0.15	18	794	86	0
96.0	3 × 0.15	19	800	56	0
101.0	12 × 0.15	20	1,000	59	2
129.5	7 × 0.15	21	1,032	101	1
154.5	3 × 0.15	22	1,042	61	15

* Inoculation: Six-hour culture of pneumococcus, Group II.

grew worse, and death occurred on the eighth day of the disease. Immediately after death 40 c.c. of cloudy fluid were aspirated from the pericardial cavity and found to be sterile by cultural tests.

Total amount of optochin given, 9.9 gm. Total amount of extract of antipneumococcus serum given in divided doses, 55 c.c. of 10-times concentrated serum, representing 550 c.c. of whole serum. No toxic symptoms referable to the optochin were observed.

Amount of optochin received per kilogram of body weight per twenty-four hours for each such period was: first period, 0.0255 gm.; second period, 0.028 gm.; third and fourth periods, 0.0255 gm.; thereafter, 0.028 gm.

In Case 2 (Hosp. No. 2705) the results of the serum study were similar to those obtained in the former. Table 4 shows that during the first twenty-four hours of administration of the drug there were fluctuations in the inhibitory and bactericidal power of the serum. Thus, the specimen obtained three hours after the first dose showed greater inhibitory power than did those obtained six and eight hours after the same dose; again, the specimens obtained three and six hours after the second dose both showed definite bactericidal power, whereas the specimen obtained eight hours after that dose showed inhibitory, but not bactericidal, action. After the first twenty-four hours the bactericidal action remained fairly constant throughout. Both this and the preceding case received comparable amounts of optochin per kilogram of body weight per twenty-four hours, and in both instances the drug was given in three large doses in the first twenty-four hours, so that they are quite comparable as regards dosage. They both showed definite fluctuations in the inhibitory and bactericidal power of the serum within the first twenty-four hours, during which time the drug was being given in relatively large doses eight hours apart. It is probable, if the doses were regulated throughout the treatment as in the first twenty-four hours in these two cases, that the fluctuations in the inhibitory and bactericidal power of the serum (and, therefore, in the concentration of the optochin in the circulating blood) would continue, although perhaps be less marked. After a period of treatment, however, owing to the retention or accumulation of optochin within the body, to be referred to later, and to its rapid absorption, such a comparatively large dose as 0.5 gm. might give rise, for a short period after its administration, to a concentration of the drug in the blood injurious to some part of the body, for example, the retina.

As declared in the foregoing, the occurrence of such fluctuations in the concentration of optochin in the blood of patients with lobar pneumonia during treatment with the drug seems undesirable.

Dosage B.—Small equal doses at regular intervals from the outset.

Several patients were treated according to this method and the details of two of these cases follow, together with the results obtained from a study of the bactericidal power of the serum in each case.

Case 3 (No. 2639).—P. C., plumber, aged 41, weight 72.8 kg.

Past History.—Alcoholic.

Present Illness.—Chill, pain in chest and cough the day before admission.

Status on Admission: temperature, 103.5 F.; pulse, 98; respirations, 30; involvement of right middle lobe; roentgenogram shows sharply circumscribed shadow in this area; white blood count, 22,000; blood culture sterile; sputum mucopurulent, blood streaked, containing pneumococcus, Group IV.

Course: Optochin started on second day of disease; optochin given at regular intervals at the rate of 11 doses of 0.15 gm. each in periods of twenty-four hours. Patient's condition improved and on the morning of the fourth day the temperature had reached 100 F., and by the fifth day it was normal. There was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 4.5 gm. No toxic symptoms referable to the optochin were observed. Amount of optochin given per kilogram of body weight per twenty-four hours, 0.0226 gm.

TABLE 5.—Bactericidal Test of Serum of Case 3 (No. 2639)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 24 Hours Inc.
0.0	1 × 0.15	1	311	8,850	C.
2.25	1 × 0.15	2	263	2,340	C.
4.5	1 × 0.15	3	320	660	C.
6.75	1 × 0.15	4	376	560	C.
9.0	2 × 0.15	5	334	332	C.
13.5	1 × 0.15	6	333	378	4,000 App.#
16.5	1 × 0.15	7	307	360	70
18.0	1 × 0.15	8	324	252	300
20.25	1 × 0.15	9	324	325	9
22.5	2 × 0.15	10	303	314	6
27.0	2 × 0.15	11	324	343	12
31.5	2 × 0.15	12	320	322	2
36.0	2 × 0.15	13	354	270	2
40.5	2 × 0.15	14	296	275	1
46.0	3 × 0.15	15	260	310	28
51.75	4 × 0.15	16	322	327	0
61.5	.....	17†	296	335	197
65.6	.....	18‡	350	503	10
72.75	.....	19§	352	397	C.
96.5	.....	20¶	297	2,000 Est.	C.

* Inoculation: 0.001 c.c. of six-hour broth culture of pneumococcus, Group II.
† Two hours after last dose.
‡ Five and one-half hours after last dose.
§ Thirteen and one-fourth hours after last dose.
¶ Thirty-nine hours after last dose.
# App. = approximately.

In Case 3 (Hosp. No. 2639), in which the amount of optochin per kilogram of body weight per twenty-four hours was 0.0226 gm., the bactericidal action appeared within 16.5 hours after the initial dose of optochin, remained practically constant for the remainder of the treatment (with the exception of specimen 8), and disappeared between 5.5 and 13.25 hours after the last dose. Previous to the appearance of the bactericidal action, a progressive increase in the temporary inhibition was observed.

Case 4 (No. 2659).—M. D., housewife, aged 29, weight 74.5 kg.

Past History.—Unimportant.

Present Illness.—Chill six days before admission, cough and pain in the chest three days before admission, headache, fever and prostration.

Status on Admission: temperature, 103.6 F.; pulse, 96; respirations, 40; involvement of the left lower lobe; white blood count, 16,000; blood culture sterile; sputum mucopurulent, containing pneumococcus, Group I.

Course: Optochin started on day of admission, sixth day of disease; patient received twelve doses of 0.15 gm. each in twenty-four hours; temperature fell by crisis seven hours after optochin was started; there was no spread of the pneumonic lesion and the affected lobe cleared up gradually.

Total amount of optochin given, 1.8 gm. No toxic symptoms referable to optochin were observed. Amount of optochin given per kilogram of body weight per twenty-four hours, 0.0241 gm.

TABLE 6.—Bactericidal Test of Serum of Case 4 (No. 2659)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 6 Hours Inc.	After 22 Hours Inc.
0.0	2 × 0.15	1	289	6,000 App.	C.	C.
2.5	.....	2	345	4,000 App.	C.	C.
4.0	1 × 0.15	3	439	4,000 App.	C.	C.
6.0	1 × 0.15	4	378	2,600	C.	C.
8.0	1 × 0.15	5	450	1,800	C.	C.
10.0	1 × 0.15	6	370	800	A. C.	C.
12.0	2 × 0.15	7	448	554	A. C.	C.
16.0	1 × 0.15	8	341	488	A. C.	C.
18.0	2 × 0.15	9	220	443	638	136
22.0	1 × 0.15	10	423	360	217	110
26.0	.....	11†	430	308	357	17
53.0	.....	12‡	279	514	A. C.	C.

* Inoculation: 0.0001 c.c. of four-hour broth culture of pneumococcus, Group II.
† Four hours after last dose.
‡ Thirty-one hours after last dose.

In Case 4 (Hosp. No. 2659) the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0241 gm. and bactericidal action was manifest within eighteen hours after the initial

dose. Previous to this time there was a steady increase in the temporary inhibitory action of the serum.

On the basis of these two preceding cases, it may be concluded that such a system of dosage gives rise to a progressive increase in the concentration of optochin in the blood stream of the patient, but, as compared with the previous system of dosage, the time of appearance of bactericidal action is somewhat delayed. In order to secure a more rapid appearance of this bactericidal action, and yet to maintain it at a constant level, or at least to avoid such fluctuations as were exhibited by the first system of dosage studied, it would seem advisable to give one comparatively large dose at the outset and follow it with numerous smaller doses at regular intervals, as illustrated in the next section.

Dosage C.—An initial comparatively large dose followed at regular intervals by smaller equal ones.

The majority of our patients were treated in this manner, and, from the standpoint of early appearance and constancy of bactericidal action in the serum, the results obtained with this system of dosage were more satisfactory than those with any of the other methods. Below are given the details of four cases so treated, together with the results of a study of the bactericidal power of the serum.

Case 5 (No. 2640).—D. C., cobbler, aged 46, weight 55.8 kg.

Past History.—Pneumonia on the right side seven years previously.

Present Illness.—Three days before admission, chill, pain in right side of chest, bloody sputum and general prostration.

Status on Admission: temperature, 104.6 F.; pulse, 110; respirations, 48; involvement of right lower lobe. Roentgenogram shows shadow in this area; white blood count 18,000; blood culture sterile; sputum tenacious, rusty and containing *Pneumococcus mucosus* (Group III).

Course: Optochin started on fourth day of the disease; one dose of 0.45 gm. and seven doses of 0.15 gm. in first twenty-four hours; thereafter ten doses of 0.15 gm. every twenty-four hours. The patient continued to have fever until the ninth day, when the temperature began to fall, reaching 99.8 F. on the tenth day of the disease. There was no spread of the pneumonic process during treatment. Subsequently fibrinous pleurisy developed on the affected side, which was accompanied by moderate fever for several days. Ultimately this condition cleared up also.

Total amount of optochin given, 9.45 gm. No toxic symptoms referable to the drug were observed. Amount of optochin in grams per kilogram of body weight per twenty-four hours, 0.0268.

Table 7 shows that in this case (Case 5, Hosp. No. 2640), in which the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0268 gm., bactericidal action appeared within 5.5 hours after the initial dose of optochin, and after the patient had received one dose of 0.45 gm. and one of 0.15 gm. This action remained constant until more than eight hours after the last dose of optochin had been administered, after which it disappeared. This experiment was repeated with another four-hour-old culture of pneumococcus and similar results were obtained.

TABLE 7.—Bactericidal Test of Serum in Case 5 (No. 2640)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 24 Hours Inc.
0.0	1 × 0.45 1 × 0.15	1	222	508	C.
5.5	1 × 0.15	2	284	247	1
8.75	1 × 0.15	3	305	282	33
12.0	1 × 0.15	4	278	310	5
14.5	1 × 0.15	5	284	269	6
17.75	1 × 0.15	6	260	280	9
20.75	1 × 0.15	7	314	284	5
23.0	4 × 0.15	8	302	261	2
34.0	3 × 0.15	9	248	253	8
41.0	2 × 0.15	10	296	292	15
46.0	5 × 0.15	11	266	278	0
58.5	4 × 0.15	12	260	308	17
69.5	5 × 0.15	13	211	264	3
91.75	10 × 0.15	14	288	264	0
106.0	10 × 0.15	15	270	265	10
130.5	4 × 0.15	16	269	261	0
139.25	5 × 0.15	17	273	288	13
153.5	1 × 0.15	18	306	255	1
162.0	.....	19†	257	318	2
187.5	.....	20‡	278	252	C.
210.0	.....	21§	249	329	C.

* Inoculation: 0.0000 c.c. of four-hour broth culture of pneumococcus, Group II.
† Eight hours after last dose.
‡ Thirty-three and one-half hours after last dose.
§ Fifty-six hours after last dose.

Case 6 (No. 2673).—H. W., business man, aged 39, weight 81.6 kg.

Past History.—Alcohol to excess.

Present Illness.—Pain in chest, cough, blood-tinged sputum and fever three days before admission.

Status on Admission: temperature, 102.4 F.; pulse, 122; respirations, 34; involvement of right lower lobe; blood culture positive—pneumococcus, Group II; sputum tenacious, blood tinged, containing pneumococcus, Group II; white blood count, 12,800.

Course: Optochin was started on the third day. In the first twenty-four hours an initial dose of 0.5 gm., followed by nine doses of 0.15 gm. each; in second twenty-four hours, thirteen doses of 0.15 gm. each; in third and fourth periods of twenty-four hours, twelve doses of 0.15 gm. each, and in fifth period of twenty-four hours, eleven doses of 0.15 gm. each. On the fourth day of the disease, after twenty-four hours of optochin, blood culture was sterile; on the fifth day physical signs indicated a spread of the pneumonic process to the right upper lobe, and the patient was definitely more ill. Optochin was continued,

and in addition extract of antipneumococcus serum, Type II, was given intravenously. The temperature reached normal on the eighth day.

Total amount of optochin given was 9.5 gm. Total amount of extract of antipneumococcus serum given, in divided doses, was 155 c.c., representing 620 c.c. of whole serum. During administration of optochin the patient developed tinnitus and partial deafness, both of which disappeared after the drug was discontinued.

Total amount of optochin given per twenty-four hours: first twenty-four hours, 1.85 gm.; second twenty-four hours, 1.95 gm.; third and fourth, 1.80 gm.; fifth, 1.65 gm.

Amount of optochin given per kilogram of body weight in the first twenty-four hours, 0.0226 gm.; in the second twenty-four hours, 0.0238 gm.; in the third and fourth twenty-four hours, 0.022 gm.; in the fifth twenty-four hours, 0.0202 gm.

TABLE 8.—Bactericidal Test of Serum in Case 6 (No. 2673)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 7 Hours Inc.	After 19½ Hours Inc.
0.0	1 × 0.5 1 × 0.15	1	584	7,800	C.	C.
4.0	2 × 0.15	2	550	672	18,000	A. C.
8.0	2 × 0.15	3	604	528	6,200	15,000 Est.
12.0	1 × 0.15	4	597	584	775	222
15.0	1 × 0.15	5	597	588	660	327
17.0	1 × 0.15	6	529	...	994	396
21.0	1 × 0.15	7	550	...	508	175
23.5	6 × 0.15	8	604	...	452	206
36.0†	1 × 0.15	9†	562	579	394	62
37.0‡	1 × 0.15	10‡	618	570	315	25
39.5	2 × 0.15	11	640	625	296	8
42.5	12 × 0.15	12	548	464	282	17
64.25	11 × 0.15	13	624	552	229	9
86.5	12 × 0.15	14	608	567	280	10
112.0	6 × 0.15	15	602	...	388	44
137.0	.....	16§	606	...	466	C.

* Inoculation: 0.001 c.c. of 6½-hour broth culture of pneumococcus, Group II.
† Immediately before first dose of extract of antipneumococcus serum intravenously.
‡ One hour after first dose of extract of antipneumococcus serum intravenously.
§ Four hours after last dose of optochin.

In Case 6 (Hosp. No. 2673, Table 8) the amount of optochin given per kilogram of body weight in the first twenty-four hours was 0.0226 gm., and bactericidal action was manifest twelve hours after the initial dose of optochin. It persisted as long as the patient continued to receive the drug (except in Specimen 6, where it was replaced by inhibition). Thirty-six hours after the initial dose of optochin, this

patient began to receive extract of antipneumococcus serum intravenously in addition to the optochin; an increase in the bactericidal power of the serum was not demonstrated after this form of treatment was instituted. Since neither the antipneumococcus serum nor the extract thereof possess pneumococcidal action, this result was not surprising.

Clinically, this case is of interest because of the fact that, although the patient on the fifth day of the disease had received optochin for thirty-six hours and his blood during twenty-four hours of that time showed a bactericidal action on pneumococci, nevertheless the physical signs indicated that the pneumonic process was spreading at the time and involving another lobe. The blood culture, however, which was positive before the administration of optochin, was sterile after twenty-four hours of optochin treatment.

Case 7 (No. 2611).—M. B., laborer, aged 60, weight 58.4 kg.

Past History.—Unimportant.

Present Illness.—Chill thirty-six hours before admission; pain in the chest, cough and blood-tinged sputum twenty-four hours before admission.

Status on Admission: temperature, 102.6 F.; pulse, 112; respirations, 36;

TABLE 9.—Bactericidal Test of Serum in Case 7 (No. 2611)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5½ Hours Inc.	After 20 Hours Inc.
0.0	1 × 0.45	1	745	9,100	C.	C.
3.0	1 × 0.15	2	744	2,300	20,000 Est.	C.
6.0	2 × 0.15	3	715	1,550	10,000 Est.	C.
12.0	1 × 0.15	4	757	1,260	4,300	10,000 Est.
15.0	1 × 0.15	5	653	1,080	2,190	894
18.0	1 × 0.15	6	681	950	2,100	455
21.0	2 × 0.15	7	695	1,140	1,700	773
26.5	3 × 0.15	8	672	984	1,580	1,700
33.5	4 × 0.15	9	694	1,210	1,700	198
42.5	1 × 0.15	10	703	1,025	917	41
46.0	1 × 0.15	11†	721	1,104	1,430	306
47.5	1 × 0.15	12‡	715	1,010	1,260	400
50.0	2 × 0.15	13	742	1,120	1,160	530
56.0	3 × 0.15	14§	627	870	1,180	62
66.0	10 × 0.15	15	703	844	550	14

* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

† Immediately before patient received first dose of 10 c.c. of extract of antipneumococcus serum subcutaneously.

‡ One and one-half hours after patient had received 10 c.c. of extract of antipneumococcus serum subcutaneously.

§ Two hours after second dose of 10 c.c. of extract of antipneumococcus serum subcutaneously.

involvement of part of right lower lobe; roentgenogram shows shadow in this area; white blood count, 11,500; blood culture sterile; sputum tenacious, hemorrhagic, containing pneumococcus, Group II.

Course: Optochin started about forty hours after chill. Schedule as follows: dosage expressed in periods of twenty-four hours each; first dose 0.45 gm., followed by seven doses of 0.15 gm.; thereafter ten doses of 0.15 gm. Forty-six hours after the beginning of the administration of optochin treatment, administration of extract of antipneumococcus serum (Group II) was commenced. A total of 40 c.c. of extract was given in divided doses, subcutaneously, representing 220 c.c. of whole serum. The disease ran a protracted course, the temperature remaining elevated and physical signs persisting for thirty days, at which time they began to clear up. For a while it was thought that the patient might have tuberculosis, but this diagnosis was not confirmed. The affected part of the lung ultimately cleared up and the temperature became normal. The condition was thought to be one of delayed resolution. There was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 5.7 gm. No toxic symptoms referable to the drug were observed. Amount of optochin given per kilogram of body weight per twenty-fours, 0.0256 gm.

In Case 7 (Hosp. No. 2611), in which the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0256 gm., bactericidal action appeared within fifteen hours after the initial dose of optochin, and, with the exception of one specimen (eighth), remained constant while the drug was being administered. This patient, also, received extract of antipneumococcus serum subcutaneously forty-six hours after the optochin was started; no increase in the bactericidal activity of the serum was observed after this treatment was begun. The figures (Table 9) show an initial increase in the number of pneumococci in the first one and one-half hours of incubation; it is followed later in most instances by a diminution. Attention was drawn previously to this phenomenon, which is not frequent.

Case 8 (No. 2566).—E. Q., man, stenographer, aged 21, weight 67.2 kg.

Past History.—Unimportant.

Present Illness.—Pain in chest, chilly sensations and blood-tinged sputum the day before admission.

Status on Admission: temperature, 102.3 F.; pulse, 96; respirations, 32; dulness with suppression of breath sounds over left lower lobe; pleuritic friction rub in this area; white blood count, 14,200; blood culture sterile; sputum tenacious, blood tinged, containing *Pneumococcus mucosus* (Group III).

Course: Optochin was started on the day of admission, a little more than twenty-four hours after the onset of the disease. Schedule as follows: in first twenty-four hours, first dose 0.45 gm., followed by seven doses of 0.15 gm.; in second twenty-four hours, ten doses of 0.15 gm., and in next 15.5 hours, six doses of 0.15 gm. The temperature remained relatively low and reached normal on the sixth day. The condition of the patient remained excellent; no increase in the extent of the signs present on admission was observed.

Total amount of optochin given, 3.9 gm. The patient complained of ringing in the ears and became slightly deaf, while optochin was being administered. Amount of optochin given per kilogram of body weight per twenty-four hours, 0.0223 gm. (first two periods).

In Case 8 (Hosp. No. 2566) the amount of optochin given in each first and second period of twenty-four hours was 0.0223 gm. Directing

TABLE 10.—Bactericidal Test of Serum in Case 8 (No. 2566)

Time in Hours After Initial Dose of Optochin	Doses of Optochin in Gm. Following Corresponding Test Bleeding	Specimen of Serum Tested	Three-Hour Old Culture. Number of Colonies of Pneumococci per 0.5 C.c. Serum When Plated*				Twenty-Four-Hour Old Culture. Number of Colonies of Pneumococci per 0.5 C.c. Serum When Plated†			
			Immediately	After 1½ Hrs. Inc.	After 5½ Hrs. Inc.	After 22 Hrs. Inc.	Immediately	After 3⅔ Hrs. Inc.	After 7⅔ Hrs. Inc.	After 20¼ Hrs. Inc.
0	1 × 0.45	1	117	2,500	C.	C.	148	10,000	C.	
3	1 × 0.15	2	101	202	5,000 Est.	C.	166	158	1,058	5,000 Est.
6	1 × 0.15	3	114	156	4,000 Est.	A. C.	169	109	202	4,000 Est.
9	1 × 0.15	4	110	130	1,680	A. C.	232	62	123	5
12	1 × 0.15	5	137	124	1,150	40	133	57	45	1
15	1 × 0.15	6	92	115	662	26	204	63	46	0
18	1 × 0.15	7	90	131	770	A. C.	174	62	64	0
21	1 × 0.15	8	127	154	531	10	199	53	42	0
24	7 × 0.15	9	115	128	661	872	166	65	52	1
42	5 × 0.15	10	106	117	400	900	210	44	30	4
54	4 × 0.15	11	106	630	A. C.	....	168	222	A. C.	C.
‡	.....	12‡	116	1,740	C.	....	195	5,000	C.	C.

* Inoculation: Broth culture of pneumococcus, Group II.
† Inoculation: Broth culture of pneumococcus, Group II.
‡ One and one-fourth hours after last dose.

attention first to the test made with the three-hour culture, it is seen that bactericidal action made its appearance within twelve hours after the initial dose of optochin (Specimen 5); it was not constantly present, however, as Specimen 7 of serum obtained eighteen hours after the first dose showed only temporary inhibition; Specimens 9 and 10, obtained twenty-four and forty-two hours, respectively, after the commencement of the drug, showed temporary inhibition likewise. One explanation which may be offered to account for the substitution of inhibitory for bactericidal action in these specimens is that the time elapsing between some of the individual doses about these periods was almost four hours (doses 6 and 7, and doses 12, 13 and 14). Such intervals between doses of 0.15 gm. may be too great to maintain the drug at a constant concentration in the circulating blood, and evidence will be presented later in support of this contention. Specimen of serum 11, obtained fifty-four hours after the initial dose of optochin and one and three-quarters hours after the immediately preceding dose was given, showed no bactericidal action and comparatively slight temporary inhibition, while that obtained one and a quarter hours after the last dose showed almost no inhibition whatever. This late disappearance of bactericidal action may possibly be accounted for, in addition, by the fact that during this time the patient was receiving

the drug at the rate of 1.23 gm. in twenty-four hours, instead of 1.5 gm., which factor would alter materially the body-weight relationship, making the amount of optochin received per kilogram of body weight per twenty-four hours only 0.018 gm. As judged by the same culture, twenty hours old, bactericidal action made its appearance nine hours after the initial dose of the drug (Specimen 4), that is, three hours earlier than when the four-hour culture was used for the test; moreover, it was constantly maintained, except in Specimens 11 and 12, in which it was replaced by temporary inhibition, as when the four-hour culture was used.

This test shows, again, the contrast between the results obtained with "old" and "young" cultures. The twenty-hour culture did not show the same fluctuation in bactericidal action as did the four-hour culture, and this action was observed three hours earlier in the case of the former.

The results obtained from a study of these three methods of dosage, with reference to time of appearance of bactericidal action, are summarized in Table 11.

TABLE 11.—Comparison of Methods of Dosage with Reference to the Time of Appearance of Bactericidal Action

Method of Dosage	Case Number	Amount of Optochin per Kilo of Body Weight in First 24 Hours, Gm.	Time of Appearance of Bactericidal Action, Hrs.
A	1 (2600)	0.0230	11.0 inconstant
	2 (2705)	0.0255	11.0 inconstant
	Average.....	0.0242	11.0
B	3 (2620)	0.0230	16.5
	4 (2689)	0.0241	18.0
	Average.....	0.0239	17.25
C	5 (2540)	0.0268	5.5
	6 (2673)	0.0226	12.0
	7 (2611)	0.0256	15.0
	8 (2566)	0.0222	12.0
	Average.....	0.0243	11.1

An analysis of Table 11 shows that the cases are quite comparable as regards the relationship of amount of optochin given in twenty-four hours to body weight of the patients. It shows, further, that, with Dosage A, the average time of appearance of bactericidal action was

eleven hours after the initial dose of optochin, and, as was seen from the protocols, that, under this system of dosage, the inhibitory and bactericidal action was inconstant during the first twenty-four hours. In the case of Dosage B, the average time of appearance of bactericidal action was 17.25 hours. With Dosage C the average time of appearance of the same was 11.1 hours, and (with the exception of Case 8, Hosp. No. 2566) it remained constant. On a basis of these facts it seems justifiable to conclude that the third method of dosage, namely, Dosage C (p. 627), is the most satisfactory one to adopt in order to obtain a bactericidal action in the serum in the shortest possible time, and to maintain it at a constant level. This conclusion is supported by further cases, given in detail later.

RELATIONSHIP BETWEEN BODY WEIGHT OF PATIENT AND APPEARANCE OF BACTERICIDAL ACTION IN THE SERUM

As stated previously, if the rates of absorption and elimination of optochin are comparable in different cases, the concentration of the drug in the blood stream might be expected to bear some relationship to the body weight of the patient; that is to say, the concentration of optochin should be greater in those cases which receive a larger quantity of the drug per kilogram of body weight than in those which receive a lesser amount. In view of these considerations, it is of interest to study the occurrence of bactericidal action in the serum with reference to the relationship to body weight of the amount of optochin given per twenty-four hours in the cases which are strictly comparable as regards method of dosage. We shall, therefore, with this idea in mind, take up a consideration of those cases in which the patients were treated according to that system of dosage which appears to be the optimum as regards spacing of individual doses and time of appearance of bactericidal action. On the basis of the results given in Table 9 it would seem that the optimum method is represented by Dosage C (p. 627), in which the patient is given a single comparatively large initial dose, followed by smaller ones at regular intervals. Sixteen patients were treated by mouth according to this method of dosage, and of these, eleven have been selected for comparison; that is, only those cases that are strictly comparable as regards spacing of doses and detail of bactericidal tests, such as age of culture and size of inoculum. The weights of these patients varied from 39.1 to 81.6 kg., and the total amount of optochin given in twenty-four hours, from 1.0 to 1.85 gm.

With reference to the relationship between the body weight of the patient and the amount of optochin received per twenty-four hours, it is possible to group the cases as follows:

1. Where the patients were of average size and the amount of optochin given to each in twenty-four hours was 1.5 gm., so that the amount

of optochin per kilogram of body weight per twenty-four hours was from 0.022 to 0.0268 gm. Three cases, 8, 7 and 5 (Hosp. Nos. 2566, 2611 and 2640) illustrate this condition, and the details of the serologic study of each have already been given (see section on dosage, Tables 10, 9 and 7). These cases show that under this system of dosage and with the relationship of body weight to amount of optochin such that the patients received 0.0223 to 0.0268 gm. per kilogram per twenty-four hours, bactericidal action was manifest in less than fifteen hours in each instance.

2. Where the patients were above average size and the total amount of optochin (1.5 gm.) given in twenty-four hours was such that the patient received less than 0.022 gm. per kilogram per twenty-four hours. Three patients were so treated; in one of them (Case 9, Hosp. No. 2581) it was impossible to demonstrate any bactericidal action at all, and in the remaining two (Hosp. Nos. 2568 and 2634) the bactericidal action was considerably delayed. In Hosp. No. 2568 the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0201 gm., and bactericidal action did not appear until between sixty-eight and ninety-two hours after the first dose; in Hosp. No. 2634 the amount of optochin given per kilogram of body weight per twenty-four hours was 0.0213 gm. and bactericidal action took twenty-six hours to appear. Below are given the details of Case 9 (Hosp. No. 2581) with the results of a study of the serum of the same (Table 12).

Case 9 (No. 2581).—T. B., watchman, aged 35, weight 80 kg.

Past History.—Unimportant.

Present Illness.—Chill and vomiting thirty-six hours before admission; pain in chest, cough and bloody sputum twenty-four hours before admission.

Status on Admission: temperature, 103.8 F.; pulse, 116; respirations, 36; involvement of right lower lobe; white blood count, 23,000; blood culture sterile; sputum tenacious, hemorrhagic, containing pneumococcus, Group II.

Course: Optochin was started by mouth on the third day of the disease; initial dose of 0.5 gm. and seven doses of 0.15 gm. each in first twenty-four hours; thereafter ten doses of 0.15 gm. in every subsequent period of twenty-four hours. The patient became steadily worse, and on the fifth day, after two and one-half days of administration, the drug was discontinued, and the patient was given extract of antipneumococcus serum, Type II, subcutaneously. Temperature began to fall on the tenth day of the disease and reached 99.8 F. on the eleventh day. In this case both the optochin and the extract of antipneumococcus serum were discontinued, as clinical observation seemed to indicate that the course of the disease was not being influenced by either.

Total amount of optochin given, 3.8 gm. Total amount of extract of antipneumococcus serum given was 106 c.c., in divided doses, representing 583 c.c. of whole serum. No toxic symptoms referable to the optochin were observed. Amount of optochin per kilo of body weight per twenty-four hours was 0.0187 gm.

Table 12 shows that the patient (Hosp. No. 2581) did not possess a bactericidal power in the blood serum at any time during the course of administration of optochin, the figures only showing temporary

TABLE 12.—Bactericidal Test of Serum in Case 9 (No. 2581)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 7½ Hours Inc.	After 15½ Hours Inc.
0.0	1 × 0.5	1	1,800	A. C.	C.
3.0	1 × 0.15	2	1,404	T. N.†	C.
6.0	1 × 0.15	3	1,356	3,500 App.	C.
9.0	1 × 0.15	4	1,078	2,600 App.	C.
12.0	1 × 0.15	5	1,580	1,620	0
15.0	1 × 0.15	6	1,640	988	C.
18.0	1 × 0.15	7	1,528	768	C.
21.0	1 × 0.15	8	1,412	1,612	C.
24.0	2 × 0.15	9	1,484	1,416	C.
30.0	2 × 0.15	10	1,436	1,774	A. C.
36.0	2 × 0.15	11	1,470	1,900	C.
42.0	6 × 0.15	12	1,330	1,488	A. C.
58.5	2 × 0.15	13	1,354	1,456	A. C.

* Inoculation: 0.00001 c.c. of an eighteen-hour broth culture of pneumococcus, Group I.
† T. N. = too numerous to count.

inhibition. The amount of optochin given per twenty-four hours per kilogram of body weight was 0.0187 gm. Clinically, the course of the disease seemed to be entirely uninfluenced by the drug. At this point it is desirable to draw attention to Case 8 (Hosp. No. 2566, Table 10), in which bactericidal action was demonstrable within twelve hours after the administration of the drug was begun, during which time optochin was being given on a basis of 0.0223 gm. per kilogram of body weight per twenty-four hours. When, toward the end of the course of administration, the amount of drug was diminished, so that the quantity given per kilogram of body weight per twenty-four hours amounted to 0.018 gm., no bactericidal action, and only a slight degree of temporary inhibition, was observed.

3. Where the patient was above average size (81.6 kg.), the amount of the drug (1.85 gm. to 1.95 gm.) given per twenty-four hours was such that the patient received from 0.0226 gm. to 0.0238 gm. of optochin per kilogram per twenty-four hours (first forty-eight hours). Such a condition was illustrated by one case (Case 6, Hosp. No. 2673), which has already been reported in detail. (See section on Dosage, Table 8.) In this case bactericidal action was manifest within twelve hours after the initial dose.

4. Where the patients were below average size and the total amount of the drug given per twenty-four hours was less than 1.5 gm., but the

quantity given per kilogram of body weight per twenty-four hours was from 0.025 to 0.0285 gm. (a relationship which in an adult of average size should, as has been seen, insure bactericidal action in the serum within twenty-four hours). Two patients were so treated. The details of the results of the bactericidal tests in these two cases are given (Tables 13 and 14).

CASE 10 (No. 2675).—J. S., schoolboy, aged 15, weight 39.1 kg.

Past History.—Unimportant.

Present Illness.—Pain in chest, cough and fever two days before admission.

Status on Admission: temperature, 104.2 F.; pulse, 112; respirations, 32; involvement of left lower lobe; white blood count, 38,000; blood culture sterile; sputum tenacious, mucopurulent, containing pneumococcus, Group IV.

Course: Optochin started on fourth day of disease; patient received during first twenty-four hours an initial dose of 0.3 gm., followed by seven doses of 0.1 gm. Within twenty-four hours after the optochin was started, crisis set in and the temperature was normal on the fifth day. There was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 1 gm. No toxic symptoms referable to the drug were observed. Amount of optochin per kilo of body weight per twenty-four hours, 0.0255 gm.

TABLE 13.—BACTERICIDAL TEST OF SERUM IN CASE 10 (No. 2675)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 6 Hours Inc.	After 23½ Hours Inc.
0.0	1 × 0.3	1	680	10,100	C.	C.
1.5	1 × 0.1	2	628	762	91,000	C.
6.5	1 × 0.1	3	641	627	6,000	C.
9.5	1 × 0.1	4	622	698	2,500	C.
12.5	1 × 0.1	5	810	688	1,700	10,000 Est.
15.5	1 × 0.1	6	676	627	848	417
18.5	1 × 0.1	7	644	680	567	35
71.5	1 × 0.1	8	666	746	520	10
74.5	.....	9	612	500	332	40

* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

CASE 11 (No. 2626).—O. H., schoolboy, aged 15, weight 42.2 kg.

Past History.—Chronic otitis media.

Present Illness.—Chill, fever, cough and blood-tinged sputum four days before admission.

Status on Admission: temperature, 104 F.; pulse, 104; respirations, 44; bilateral otitis media with perforation of both drums and partial deafness; involvement of left lower lobe; white blood count, 15,000; blood culture sterile; sputum mucoid, blood tinged, containing pneumococcus, Group II.

Course: Optochin started on the sixth day of the disease; patient given 0.3 gm., then nine doses of 0.1 gm. each, making 1.2 gm. in first twenty-four hours; then twelve doses of 0.1 gm. each in second twenty-four hours, and eight doses of

0.15 gm. each in succeeding periods of twenty-four hours. Temperature showed morning remissions on eighth, ninth and tenth days and reached normal on the eleventh day of the disease. There was no spread of the pneumonic process to any other lobe. Lung cleared up slowly.

Total amount of optochin given, 5.65 gm. During the administration of the drug the patient became almost completely deaf, but this condition receded to that present on admission when the optochin was discontinued. Amount of optochin per kilo of body weight per twenty-four hours, 0.0284 gm.

TABLE 14.—Bactericidal Test of Serum of Case 11 (No. 2626)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 23 Hours Inc.
0.0	1 × 0.3	1	600	3,870	C.
2.5	1 × 0.1	2	714	723	C.
5.0	1 × 0.1	3	719	792	C.
7.5	1 × 0.1	4	928	1,084	C.
10.0	3 × 0.1	5	726	767	C.
17.75	3 × 0.1	6	690	767	A. C.
24.5	3 × 0.1	7	796	701	1
31.0	6 × 0.1	8	700	708	1
42.75	4 × 0.1	9	720	680	4
52.75	4 × 0.1	10	648	705	12
72.75	11 × 0.1	11	677	620	3

* Inoculation: 0.0005 c.c. of four-hour broth culture of pneumococcus, Group II.

A study of Tables 13 and 14 shows that in Case 10 (Hosp. No. 2675), where the amount of optochin per kilogram of body weight per twenty-four hours was 0.0255 gm., bactericidal action was present within 15.5 hours after the initial dose, although this patient received a total of only 1.0 gm. in twenty-four hours; and that in Case 11 (Hosp. No. 2626), where the amount of optochin received per kilogram per twenty-four hours was 0.0284 gm., the serum acquired a bactericidal action between 17.5 and 24.5 hours after the initial dose, although the total amount of optochin given in twenty-four hours was only 1.2 gm.

5. Finally, where patients are of average size but the quantity of drug is increased over that usually given, so that the amount received per kilogram per twenty-four hours is more than 0.028 gm. Two cases so treated are available for study. One of these (Hosp. No. 2638, Case 17) is recorded in detail later in connection with another question, and reference to Table 22 will show that, in this case, where the amount of optochin per kilogram of body weight per twenty-four hours was 0.031 gm., bactericidal action was present within eleven hours after the initial dose. The details of the other case follow immediately (Table 15).

CASE 12 (No. 2711).—A. S., tinsmith, aged 21, weight 57.4 kg.

Past History.—Unimportant, except that he drank beer freely.

Present Illness.—Chill and vomiting two days before admission. Cough and blood-tinged sputum day before admission.

Status on Admission: temperature, 104.8 F.; pulse, 104; respirations, 36. Beginning involvement of left lower lobe; white blood count, 20,000; blood culture sterile; sputum tenacious, hemorrhagic, containing pneumococcus, Group I.

Course: On day following admission the patient was more toxic and physical signs at this time indicated beginning involvement of the left upper lobe, which was borne out by examination the next day. Optochin was started on the fourth day of the disease. A blood culture taken immediately before commencement of the optochin showed the presence of three colonies of pneumococcus, Group I, per cubic centimeter of blood. The patient received in the first twenty-four hours one dose of 0.45 gm., followed by eight doses of 0.15 gm. each at regular intervals. Thereafter he received ten doses of 0.15 gm. each, at regular intervals, in periods of twenty-four hours each. On the fifth day of the disease, twenty-four hours after the commencement of the optochin, the patient received the first of four intravenous injections of antipneumococcus serum (Group I), 75.0 c.c. each, given at intervals of nine to twelve hours. The temperature remained approximately constant until the eighth day of the disease, when it fell below 100 F. The lungs cleared slowly.

Total amount of optochin given, 4.05 gm. Total amount of antipneumococcus serum (Group I), 300 c.c. No toxic symptoms referable to the optochin were observed. Amount of optochin per kilo of body weight, 0.0287 gm. first twenty-four hours; thereafter, 0.026 per twenty-four hours.

TABLE 15.—BACTERICIDAL TEST OF SERUM OF CASE 12 (HOSP. No. 2711)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5 Hours Inc.	After 21 Hours Inc.
0.0	1 × 0.45	1	188	200	2,000	C.
2.0	1 × 0.15	2	108	165	159	79
4.0	1 × 0.15	3	122	162	158	4
6.0	1 × 0.15	4	170	188	109	4
9.0	1 × 0.15	5	153	142	251	39
12.0	1 × 0.15	6	135	161	139	8
15.0	1 × 0.15	7	168	184	124	66
18.0	1 × 0.15	8	139	178	151	199
21.0	1 × 0.15	9	141	97	160	706
23.0†	.....	10	122	137	201	168
24.0	4 × 0.15	11	131	161	180	1,015
32.5†	15 × 0.15	12	101	170	110	14

* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.
† Indicates that at this point the patient was given 75 c.c. of antipneumococcus serum (Group I) intravenously immediately after the corresponding test bleeding.

In Case 12 (Hosp. No. 2711) the amount of optochin per kilogram of body weight given in the first twenty-four hours was 0.0287 gm. and bactericidal action was manifest within two to four hours after the

initial dose. The culture used in this test was not growing very actively; this was afterwards discovered to be due to the fact that the acidity of the broth used was too high. After the patient had received the drug for fifteen hours, complete inhibition rather than bactericidal action was present (Specimens 8 to 11, inclusive). The reason for this decrease in the bactericidal activity of the patient's serum is not apparent; however, bactericidal action was again manifest within 32.5 hours after the first dose of optochin (Specimen 12). It should be noted that a blood culture taken twenty-four hours after the initial dose of optochin showed three colonies of pneumococcus, Group I, in 6 c.c. of blood; reference to Table 15 shows that at this time bactericidal action had been supplanted by mere inhibitory action.

The eleven cases that have been selected for a study of the influence of the factor of body weight on the appearance of bactericidal action are summarized in Table 16, where the cases are arranged in the order of the amount of optochin given per kilogram of body weight per twenty-four hours (first twenty-four hours).

TABLE 16.—Relationship Between Amount of Optochin per Kilogram of Body Weight per Twenty-Four Hours to Time of Appearance of Bactericidal Action

Hospital and Case No.	Weight, Kg.	Total Amount Optochin in First 24 Hours, Gm.	Amount of Optochin per Kilo Body Weight in First 24 Hours, Gm.	Time of Appearance of Bactericidal Action, Hrs.
9 (2581)	80	1.5	0.0187	No bactericidal action
13 (2508)	74.4	1.5	0.0201	68-92
(2634)	70.3	1.5	0.0213	26
8 (2566)	67.2	1.5	0.0223	12
6 (2673)	81.6	1.86	0.0226	12
7 (2611)	58.4	1.5	0.0256	15
10 (2675)	39.1	1.0	0.0255	15.5
5 (2640)	55.8	1.5	0.0268	5.5
11 (2626)	42.2	1.2	0.0284	17.5-24.5
12 (2711)	57.4	1.65	0.0287	4
17 (2638)	42.8	1.35	0.0315	11

A study of Table 16 shows that those patients who received less than 0.022 gm. of optochin per kilogram of body weight per twenty-four hours either did not acquire in their serum any bactericidal action, or else its appearance was delayed more than twenty-four hours from the commencement of the optochin treatment; whereas, the serum of those patients who received more than 0.022 gm. of optochin per kilo-

gram of body weight per twenty-four hours in no instance failed to show definite bactericidal properties. Furthermore, in those patients receiving more than 0.022 gm. of the drug per kilogram of body weight per twenty-four hours, the average time of appearance of the bactericidal action in the serum was 12.4 hours after the giving of the first dose.

An analysis of all our cases in which the patients were treated by mouth, irrespective of the spacing and size of individual doses, shows that in no instance did bactericidal action fail to be manifest when the patients received as much as 0.024 gm. of optochin per kilogram of body weight per twenty-four hours, with one exception (Hosp. No. 2689), where too great an interval—eight hours—elapsed between the first and second doses (see Table 31). We feel justified in concluding, therefore, that in administering optochin to human beings by mouth it is essential that the body weight of the patient be taken into consideration if the production of bactericidal action in the serum is to be insured. Individual variations in absorption and elimination are to be expected, as will be indicated later, but in the cases studied no great differences in these factors have been observed; indeed, it is rather surprising that the relationship between body weight and amount of optochin given per twenty-four hours bears such a constant relationship to the occurrence of bactericidal action in the patient's serum.

II. COMBINED INTRAMUSCULAR AND ORAL ADMINISTRATION

Table 16 shows that when optochin is given to human beings by mouth, in accordance with a definite system of regulation of doses and in such quantity that the patients receive at least 0.022 gm. of the drug per kilogram per twenty-four hours, the serum of such persons rapidly acquires (average 12.4 hours) a definite bactericidal action for the pneumococcus *in vitro*. It would seem that such an effect might be secured within a shorter space of time if the first few doses were given intramuscularly. Optochin hydrochlorid is soluble in distilled water, less soluble in physiologic salt solution. When given subcutaneously it may lead to painful infiltration. In one patient in whom it was injected too superficially, a slough was produced at the site of injection. When given intramuscularly it does not seem to be irritant.

Three patients (Hosp. Nos. 2658, 2593 and 2604) received one or more doses by the intramuscular route at the outset of the treatment, and subsequent doses by mouth. In each case the size and spacing of the individual doses conformed to what we believe to be the optimum method, and the amount of the drug given was such that in the first twenty-four hours each patient received 0.022 gm. or more per kilogram

of body weight, an amount which, judging from the results obtained from oral administration, should give rise to the production of a bactericidal action in the patient's serum within twenty-four hours.

CASE 13 (No. 2658).—J. E., manager, aged 29, weight 66.6 kg.

Past History.—Unimportant.

Present Illness.—Chill, pain in chest, cough, blood-tinged sputum day before admission.

Status on Admission: temperature, 102.5 F.; pulse, 120; respirations, 28; involvement of right lower lobe; white blood count, 47,000; sputum tenacious, blood tinged, containing pneumococcus, Group II A; blood culture sterile.

Course: Optochin started day of admission, second day of disease. Patient was given 0.5 gm. intramuscularly, then four doses of 0.25 gm. intramuscularly, all dissolved in physiologic salt solution, making 1.5 gm. in the first twenty-four hours; then ten doses of 0.15 gm. each in powder form by mouth during the succeeding periods of twenty-four hours. The temperature became normal on the fifth day of the disease and there was no spread of the pneumonic process to any other lobe.

Total amount of optochin given, 3.9 gm. No toxic symptoms referable to the optochin were observed. Amount of optochin per kilo of body weight per twenty-four hours was 0.0225 gm.

TABLE 17.—BACTERICIDAL TEST OF SERUM IN CASE 13 (No. 2658)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 6 Hours Inc.	After 20 Hours Inc.
0.0	1 × 0.5 †	1	288	12,000	5,000,000 Est.	C.
2.0	.....	2	232	901	2,000,000 Est.	C.
4.0	.....	3	402	548	500,000 Est.	C.
6.0	.....	4	350	495	500,000 Est.	C.
8.0	1 × 0.25†	5	242	621	A. C.	C.
10.0	.....	6	372	334	10,000 Est.	C.
12.0	1 × 0.25†	7	367	388	10,000 Est.	C.
14.0	.....	8	309	387	1,035	5,000
16.5	1 × 0.25†	9	304	423	2,000	2,200
18.0	.....	10	419	462	968	191
20.0	1 × 0.25† 2 × 0.15‡	11	445	485	970	1,124
28.5	2 × 0.15‡	12	402	472	404	9
26.0	2 × 0.15‡	13	377	478	316	10
40.5	2 × 0.15‡	14	402	473	220	1
47.5	2 × 0.15‡	15	350	426	341	3
52.0	4 × 0.15‡	16	430	497	300	6
		17	438	878	301	

* Inoculation: 0.001 c.c. of six-hour broth culture of pneumococcus, Group II.
† Intramuscularly. ‡ Orally.

In Case 13 (Hosp. No. 2658), in which the amount of optochin per kilogram of body weight per twenty-four hours was 0.0225 gm., bactericidal action was present in only one of eleven specimens of serum obtained during twenty-four hours of intramuscular administration of the drug, namely, in specimen 10 (eighteen hours after the commencement of the treatment); it was no longer present in the specimen taken (Specimen 11) two hours afterwards. Indeed, with the one exception of Specimen 10, bactericidal action only became manifest after oral administration had been begun; in other words, between twenty and twenty-eight and one-half hours after the initial dose. Previous to this, and with the exception of Specimen 10, the serum only showed temporary inhibition.

Two patients received initial doses of the drug, dissolved in distilled water, intramuscularly, all subsequent doses being given by mouth. The details of these cases follow.

Case 14 (No. 2593).—F. B., salesman, aged 37, weight 53.8 kg.

Past History.—Unimportant.

Present Illness.—Cold for a week before admission. Pain in chest day before admission. No chill or other symptoms.

Status on Admission: temperature, 102.4 F.; pulse, 98; respirations, 36; involvement of right lower lobe; white blood count, 12,500; blood culture sterile; sputum tenacious, rusty, containing *Pneumococcus mucosus* (Group III).

Course: Optochin started on second day of disease. A first dose of 0.4 gm. dissolved in 8 c.c. of distilled water was given intramuscularly; all subsequent doses were given by mouth. Following the first dose the patient received seven doses of 0.15 gm. each, so spaced that at the end of the first twenty-four hours he had received 1.45 gm., including the dose given intramuscularly. On account of ringing in the ears, the dosage was reduced in the second twenty-four hours, but was subsequently increased. The schedule was as follows: Dosage expressed in periods of twenty-four hours each, beginning with the second twenty-four hours; six doses of 0.15 gm., nine doses of 0.15 gm., eight doses of 0.15 gm. and ten doses of 0.15 gm. for the remainder of the treatment. The condition of the patient grew slowly but steadily worse. The involved area increased in extent until the entire right lung was affected. On the ninth day the left upper lobe showed signs of involvement, and death took place on this day. Blood culture taken eight hours before death showed thirteen colonies of *Pneumococcus mucosus* per cubic centimeter of blood. Optochin had to be discontinued thirty hours before death, owing to the inability of the patient to swallow. The patient developed ringing in the ears and partial deafness while receiving the drug.

Total amount of optochin given, 7.9 gm. Amount of optochin per kilo of body weight, first twenty-four hours, 0.0268 gm.; second twenty-four hours, 0.0167 gm.; third twenty-four hours, 0.025 gm.; fourth twenty-four hours, 0.0223 gm.; thereafter per twenty-four hours, 0.0278 gm.

In Case 14 (Hosp. No. 2593), in which the quantity of drug administered within the first twenty-four hours was 1.45 gm. (of which the first dose of 0.4 gm. was given intramuscularly), equivalent to 0.0269 gm. per kilogram of body weight, no bactericidal action was manifest within the first twenty-four hours; nor was there any considerable inhibition seen in the four hours following the first dose,

which was given intramuscularly. During the second twenty-four hours of administration the patient showed no bactericidal action, but only temporary inhibition, as might be expected, since during this period only 0.9 gm. was given, which means 0.0167 gm. per kilogram; nor did either of the two specimens tested after this time show more than complete inhibition. As no further specimen was tested after No. 13, it is impossible to state whether or not bactericidal action appeared in the serum of this patient during the remaining days of the administration of the drug. In spite of the specific treatment the pneumonic process was not controlled and the patient died.

TABLE 18.—BACTERICIDAL TEST OF SERUM OF CASE 14 (No. 2593)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
			Immediately	After 1½ Hours Inc.	After 22 Hours Inc.
0.0	1 × 0.4†	1	330	3,800	C.
1.5	.....	2	421	2,000	C.
2.5	.....	3	380	1,548	C.
4.0	1 × 0.15‡	4	400	1,264	C.
7.0	1 × 0.15‡	5	438	978	C.
10.0	2 × 0.3‡	6	409	552	C.
16.0	2 × 0.3‡	7	398	570	904
22.0	1 × 0.15‡	8	422	732	1,000 App.
27.0	3 × 0.15‡	9	470	688	2,000 App.
39.0	3 × 0.15‡	10	440	498	2,000 App.
48.0	1 × 0.15‡	11	407	484	A. C.
51.0	5 × 0.15‡	12	387	464	3,000 App.
63.75	10 × 0.15‡	13	354	470	650

* Culture used for inoculation: 0.001 c.c. of four-hour culture of pneumococcus, Group II.
† Intramuscularly. ‡ Orally.

CASE 15 (No. 2604).—D. del V., elevator operator, aged 20, weight 55.4 kg.

Past History.—Unimportant.

Present Illness.—Chill, fever, cough, pain in chest and bloody sputum two days before admission.

Status on Admission: temperature, 104.6 F.; pulse, 138; respirations, 36. Mental confusion; complete consolidation of left lower lobe; white blood count, 13,000; blood culture sterile; sputum tenacious, rusty and containing pneumococcus of Group II A.

Course: Optochin was started on fourth day of disease. Patient was given a first dose of 0.45 gm. dissolved in 9 c.c. of distilled water intramuscularly; all subsequent doses were given by mouth. Following the first dose the drug was given in divided doses, so that at the end of the first twenty-four hours he had received seven doses of 0.15 gm. in addition to the first dose of 0.45 gm., making 1.5 gm. in all. Thereafter the drug was given at the rate of 10 doses of 0.15 gm. in twenty-four hours. When the patient had received optochin for forty-eight

hours he was given 2 c.c. of extract of antipneumococcus serum, Type II, intravenously, and later 20 c.c. of the same serum in two doses subcutaneously. Temperature reached normal on the seventh day of the disease after three days' administration of the drug.

Total amount of optochin given, 4.5 gm. Total amount of extract of antipneumococcus serum (Group II) given, 22 c.c., representing 111 c.c. of whole serum. No toxic symptoms referable to the drug were observed. Amount of optochin per kilo of body weight per twenty-four hours, 0.0270 gm.

TABLE 19.—Bactericidal Test of Serum of Case 15 (No. 2604)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5½ Hours Inc.	After 24 Hours Inc.
0.0	1 × 0.45†	1	476	11,500	C.	C.
1.5	.....	2	441	11,000 Est.	C.	C.
2.5	.....	3	397	8,000	C.	C.
4.0	1 × 0.15‡	4	442	5,000	A. C.	C.
6.5	1 × 0.15‡	5	488	5,000	A. C.	C.
9.5	2 × 0.15‡	6	296	1,300	A. C.	C.
16.0	2 × 0.15‡	7	415	890	2,200	2,000 Est.
22.0	2 × 0.15‡	8	470	894	5,000	2,000 Est.
28.0	5 × 0.15‡	9	548	692	2,800	A. C.
42.0	3 × 0.15‡	10	416	701	542	27
49.5	1 × 0.15‡	11§	382	402	1,300	88
49.5	1 × 4.15¶	12¶	411	602	1,000	634
53.5	5 × 0.15‡	13	436	614	1,000	24
66.6	2 × 0.15‡	14#	402	478	654	140

* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.
† Intramuscularly.
‡ Orally.
§ Immediately before 2 c.c. of extract of antipneumococcus serum, Group II, intravenously.
¶ Immediately after the above dose of extract, and immediately before 10 c.c. of the same extract subcutaneously.
# Nine hours after a second dose of 10 c.c. of extract of antipneumococcus serum, Group II, subcutaneously.

Table 19 shows that in this case (Case 15, Hosp. No. 2604), in which the quantity of drug administered was 1.5 gm. per twenty-four hours (of which the first dose of 0.45 gm. was given intramuscularly), equivalent to 0.270 gm. per kilogram of body weight, the bactericidal action did not appear in the serum until somewhere between twenty-eight and forty-two hours after the initial dose. Here again the temporary inhibition following the intramuscular injection was by no means marked.

From the three foregoing cases it would appear that the intramuscular route is less satisfactory than the oral route.

ABSORPTION AND ELIMINATION

Some knowledge of the rates of absorption and of elimination or destruction of optochin is desirable from a therapeutic standpoint, in order that the intervals between individual doses be not so long as to permit the bactericidal action to disappear from the circulating blood. Methods for the quantitative determination of the small amounts of optochin involved are not available, so that for information needed on this point recourse must be had to a biologic method, such as that employed in this investigation.

We have seen that optochin is rapidly absorbed from the gastrointestinal tract, so that within less than three hours after 0.5 gm. has been taken by mouth it is possible to detect the specific inhibitory action in the patient's blood. Table 3 shows that at the end of six hours after such a dose the inhibitory action was greater than at the end of eight hours, which indicates that the drug at this latter time was being eliminated or destroyed. When, now, a second dose of 0.5 gm. was given by mouth, tests conducted at the end of eight hours after this dose showed a greater inhibitory or bactericidal action than those at the end of the first eight hours. Similar results are seen in Table 4. These facts indicate that, under such conditions of dosage, the rate of absorption of the drug is faster than that of elimination or destruction, as a result of which a positive balance is established and a certain amount of the drug retained in the body. When numerous comparatively small doses of the drug are given in twenty-four hours (for example, 0.15 gm. every two and one-half hours) a steady increase is observed in the inhibitory power of the blood until bactericidal action is manifest, as shown by Tables 5 and 6. This again indicates that part of the drug is being retained in the body. Such retention lasts only so long as the drug continues to be supplied in sufficient quantity, for when it is discontinued the blood soon loses its bactericidal power. Wright[4] has shown that the drug is eliminated in the urine. It is possible that it may be in part destroyed in the body. Elimination probably proceeds at a fairly rapid rate, as indicated above, hence it is important, in the treatment of patients, that the intervals between the individual doses should not be too great. When the patient is receiving numerous doses of 0.15 gm. each and has in his blood a bactericidal action, it may happen that, if four hours or more are allowed to intervene between the individual doses, the bactericidal action may disappear. The following case illustrates this point.

CASE 16 (No. 2582).—S. S., tobacconist, aged 48, weight 45.3 kg.

Past History.—Unimportant.

Present Illness.—Chill, pain in chest, cough and bloody sputum two days before admission.

Status on Admission: temperature 102.2 F., pulse 90; respirations 24. Signs

indicating beginning involvement of right upper lobe; blood culture sterile; white blood count 13,400; sputum tenacious, rusty, containing *Pneumococcus mucosus* (Group III).

Course: Optochin was started on the third day of the disease; all doses were given by mouth. The patient was given 0.4 gm. as initial dose and six doses 0.15 gm. each in first 24 hours, ten doses 0.15 gm. each in second and third twenty-four hours; the drug was then omitted for thirteen and one-half hours and then resumed as follows: in periods of twenty-four hours each, 0.45 gm. + 8 x 0.15; 7 x 0.15; 6 x 0.15; 5 x 0.15, in sixteen hours making a total of 8.65 gm. in all. Two days after admission and thirty-six hours after optochin was started, signs of consolidation of right upper lobe became well marked. The general condition of the patient grew worse for several days after optochin was started, respirations reaching 62 per minute, and he appeared extremely ill. The pneumonic process did not spread to any other lobe, however, and on the ninth day the temperature began to fall, reaching 99.8 F. on the eleventh day, when the optochin was discontinued. Physical signs on this day indicated that resolution

TABLE 20.—Bactericidal Test of Serum of Case 16 (No. 2582)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 3 Hours Inc.	After 7½ Hours Inc.	After 21½ Hours Inc.
0.0	1 × 0.4	1	567	6,346	C.	C.
1.75	1 × 0.15	2	515	277	242	16
4.75	1 × 0.15	3	569	263	244	13
7.75	1 × 0.15	4	557	383	221	35
12.0	1 × 0.15	5	603	304	209	88
15.75	1 × 0.15	6	490	338	386	2,800
19.75	1 × 0.15	7	588	449	723	5,000
23.75	4 × 0.15	8	476	486	953	A. C.
35.75	6 × 0.15	9	467	381	328	39
47.75	1 × 0.15	10	500	227	75	1
50.75	3 × 0.15	11	562	288	74	0
59.75	4 × 0.15	12	536	235	60	1
67.75†	2 × 0.15	13	486	261	65	0
	1 × 0.45 8 × 0.15					
109.0	6 × 0.15	14	264	97	7	0
132.25	6 × 0.15	15	335	96	16	0
156.25	5 × 0.15	16	272	85	11	0
177.0	.....	17‡	574	132	29	2
184.0	.....	18§	269	181	291	83
206.0	.....	19¶	259	1,000	C.	C.

* Inoculation: 0.00001 c.c. of a nineteen-hour broth culture of pneumococcus, Group II.
† Optochin stopped for thirteen and one-half hours, then resumed.
‡ Three hours after last dose.
§ Ten hours after last dose.
¶ Thirty-two hours after last dose.

was in progress. The patient became slightly deaf while receiving the drug, but this condition disappeared when the drug was stopped.

Amount of optochin per kilo of body weight per twenty-four hours; first twenty-four hours = 0.0286 gm.; second and third twenty-four hours = 0.033 gm.

It will be seen from a study of Table 20 that this patient (Case 16, Hosp. No. 2582) received an initial dose of 0.4 gm. and that within two hours his serum had acquired bactericidal power. The second dose, 0.15 gm., was given two hours after the first, and there then followed two doses of 0.15 gm. at intervals of three hours, during which time the serum was constant in its bactericidal activity. During the remaining three fourths of the first twenty-four hours the doses of 0.15 gm. were given at intervals of from three and three-fourths hours to four and one-half hours, and it is significant that between the twelfth hour and the twenty-fourth hour bactericidal action disappeared and was replaced by only slight inhibition (Specimens 6, 7 and 8). From the twenty-fourth to the thirty-sixth hour the drug was given in doses of 0.15 gm. every two or three hours, and the specimen of serum obtained about the thirty-sixth hour of treatment showed definite bactericidal activity. In this patient it would seem that, early in the course of treatment, the rates of absorption and elimination were comparatively rapid, and, also, that a period of four hours constituted too long an interval between individual doses of 0.15 gm. each.

Case 8 (Hosp. No. 2566), Table 10, illustrates the same principle. Hence, in administering frequent small doses of optochin to patients, if it be desired to establish and maintain a constant bactericidal action in the serum, too great an interval of time must not elapse between single doses.

In Case 16 (Hosp. No. 2582), Table 20, the protocol shows that after the patient had received optochin for more than seven days a specimen of serum obtained ten hours after the last dose still showed bactericidal action. This would suggest that when adequate administration of the drug lasts over a comparatively long time, considerable accumulation of the drug may take place, so that bactericidal action in the serum is maintained for a longer time than would be expected after a shorter course of administration according to the same dosage. A similar observation has been made in several of our cases. The possibility occurs, therefore, of diminishing the dosage after several days' administration of the drug, and still maintaining the bactericidal action in the serum.

In Case 6 (Hosp. No. 2673, Table 8), however, in the last twenty-four hours of treatment, that is on the fifth to sixth day, the amount of the drug given per kilogram of body weight was reduced to 0.016 gm., and the bactericidal action disappeared from the serum four hours after the last dose.

From a therapeutic standpoint it is of importance to know whether or not optochin can pass from the blood through the walls of the capillaries of the lung into the alveolar spaces. No data are available on this point. That the drug is capable of passing through the capillary walls into the serous sacs, however, is indicated by observations made on one case, Case 2 (Hosp. No. 2705, Table 4). This patient received optochin over a period of seven days and in such amounts that a decided bactericidal action became manifest in the serum within twenty-four hours after the initial dose, and remained constant throughout the course of treatment. In spite of this fact the disease process was not permanently arrested and the patient died. Immediately after death, 40 c.c. of turbid yellow fluid were aspirated from the pericardial cavity. This fluid clotted shortly after removal and was found by cultural tests to be sterile. The serum was removed from the clot and tested in the usual manner for bactericidal action on the pneumococcus. As controls, normal human serum from another patient, ascitic fluid from a patient with cirrhosis of the liver and blood serum from the patient herself obtained at the height of administration of the drug, were used. The patient's own blood serum before optochin was administered had already been shown to possess no bactericidal action for the pneumococcus. The results of the experiment are given in Table 21.

TABLE 21.—Bactericidal Test of Pericardial Fluid Obtained from Case 2 (No. 2705) (Table 4)

Fluid	Number of Colonies of Pneumococci per 0.5 C.c. When Plated*			
	Immediately	After 1½ Hours Inc.	After 5 Hours Inc.	After 21 Hours Inc.
Normal blood serum	128	780	4,000 Est.	C.
Ascitic fluid	187	688	1,500 Est.	C.
Blood serum, Case 2 (Hosp. No. 2705) (optochin for six days by mouth)	104	153	23	0
Pericardial fluid, Case 2 (Hosp. No. 2705), immediately after death	122	104	23	0

* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

Table 21 shows that the pericardial fluid obtained from the patient, Case 2 (Hosp. No. 2705) immediately after the death of the patient, and the blood serum of the same patient obtained about twenty-four hours before death and after optochin had been given by mouth for six days, both showed marked bactericidal activity for the pneumococcus; whereas, normal blood serum and ascitic fluid obtained from a case of cirrhosis of the liver showed no such bactericidal activity. It would seem justifiable to conclude from these observations that opto-

chin is capable of passing from the blood, through the capillary wall, into the pericardial cavity. This fact is of importance as regards the permeability of the capillaries for optochin and also because of the bearing it has on the possible influence of the drug on the occurrence of empyema or pericarditis in cases of lobar pneumonia.[26]

"FASTNESS"

As stated previously, pneumococci, when subjected to the action of concentrations of optochin not sufficiently great to destroy them, may react against the drug in such a manner that they attain a condition in which they are uninfluenced by concentrations of the drug formerly great enough to bring about their death. This state of resistance or "fastness" may be acquired within the animal body (mice) as well as in the test tube. In lobar pneumonia the conditions may be not unfavorable for the acquisition of this characteristic by the pneumococci. Thus, in the consolidated portions of the lung the organisms may be protected mechanically from the action of the full strength of the circulating drug, by reason of a possible relative impermeability of the exudate for optochin, or other factors.

In one of our cases in which the patient was treated consistently and according to what would seem to be the optimum method of dosage, it was possible to recover from the blood immediately after death a strain of pneumococcus which was definitely "fast" toward the drug; whereas the strain recovered from the blood before optochin treatment was begun did not exhibit this characteristic. The details of this case follow.

CASE 17 (No. 2638).—C. G., pedler, aged 55, weight 42.8 kg.

Past History.—Unimportant.

Present Illness.—Cough for several days; became worse two days before admission, when patient began to have pain in chest.

Status on Admission: temperature, 103.8 F.; pulse, 106; respirations, 40; involvement of part of right lower lobe; roentgenogram shows shadow in this area; white blood count, 18,000; sputum tenacious, slightly blood streaked, containing pneumococcus, Group IV. Blood culture showed pneumococcus, Group IV.

Course: Optochin started on the fourth day of disease; all doses given by mouth. During the first twenty-four hours the patient received a first dose of 0.5 gm., a second dose of 0.15 gm. and seven doses of 0.1 gm. each. In the second, third and fourth periods of twenty-four hours each he received twelve doses of 0.1 gm.; during the fifth period, seven doses of 0.1 gm. each and three of 0.15 gm. each; during the sixth period, nine doses of 0.15 gm. each. On the seventh day of the disease (third day of treatment) the involved area began to increase in extent and on the ninth day the entire right lower lobe was consolidated. At this time the patient was quite ill, but shortly afterward he seemed to improve somewhat, and on the tenth day of the disease the lowest temperature was 99.4 F.; the highest 101.6 F. On the eleventh day (seventh day of treatment)

26. Since the foregoing was written we have been able to demonstrate pneumococcidal action in the pericardial fluids obtained from two other patients who had received optochin hydrochlorid by mouth for several days.

the temperature was 99.2 F. (rectal). The drug was discontinued. On the following day the temperature rose again and the patient's condition grew rapidly worse. At this time physical signs indicated involvement of the left lower lobe. Optochin was immediately resumed (after an intermission of seven and one-half hours) as follows: 0.5 gm., followed by two doses of 0.1 gm. each. The patient died rather suddenly that same day (twelfth day of disease). The physical signs in the right lung indicated that at that time the pneumonic process was beginning to resolve. A culture of the heart's blood taken immediately after death showed pneumococcus, Group IV, and a strain of pneumococcus belonging to the same immunologic group was recovered from the left lower lobe at the same time. No pneumococcus was obtained by aspiration of the right lower lobe.

Total amount of optochin given, 8.75 gm. No toxic symptoms referable to the drug were observed. Amount of optochin per kilo per twenty-four hours, 0.031 gm. first twenty-four hours, 0.028 gm. second, third and fourth periods.

TABLE 22.—Bactericidal Test of Serum in Case 17 (No. 2638)

Time in Hours after Initial Dose of Optochin	Doses of Optochin in Grams Following Corresponding Test Bleeding	Specimen of Serum Tested	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*			
			Immediately	After 1½ Hours Inc.	After 5½ Hours Inc.	After 22½ Hours Inc.
0.0	1 × 0.5	1	305	7,100	C.	C.
2.0	1 × 0.15	2	231	273	15,000 Est.	C.
6.0	1 × 0.1	3	257	308	305	2,000 App.
9.0	1 × 0.1	4	288	281	295	484
11.0	1 × 0.1	5	234	221	237	89
13.0	2 × 0.1	6	262	256	285	275
16.5	1 × 0.1	7	230	226	...	10
21.0	2 × 0.1	8	230	267	...	11
25.5	2 × 0.1	9	312	319	303	7
29.5	2 × 0.1	10	233	319	280	3
33.75	5 × 0.1	11	360	322	325	4
48.5	2 × 0.1	12	314	258	175	3
47.5	3 × 0.1	13	224	231	206	1
53.5	9 × 0.1	14	268	285	231	1
71.0	12 × 0.1	15	226	238	157	35
97.0	7 × 0.1 4 × 0.15	16	334	294	206	2
123.0	8 × 0.15	17	273	227	180	3
147.5	4 × 0.15 1 × 0.5 2 × 0.1	18	348	223	181	1

* Inoculation: 0.001 c.c. of four-hour broth culture of pneumococcus, Group II.

In Case 17 (Hosp. No. 2638) the amount of drug given per kilogram of body weight for the first twenty-four hours was 0.031 gm., and later 0.028 gm.; bactericidal action was manifest eleven hours after the initial dose, and remained constant thereafter throughout the course of administration; in spite of this fact, however, the pneumonic

process spread to previously uninvolved portions of the lung and the patient died. The blood culture on admission showed the presence of pneumococcus, Group IV. Unfortunately, no other blood cultures were made during life, but a culture from the heart's blood, made immediately after death, yielded an organism belonging to the same immunologic group as did a culture from the left lower lobe (the advancing lesion) made at the same time. A culture made from the lesion in the right lung, just after death, was sterile. In all, four strains belonging to the same immunologic group were obtained from this patient (including those isolated from the sputum and blood on admission). All four strains were tested at the same time with regard to the presence or absence of "fastness" toward optochin. This was done (1) by observing the growth of the various strains in varying concentrations of the drug in nutritive bouillon, and (2) by comparing their behavior in normal serum with that in serum obtained from the patient at the height of the administration of optochin, when his serum possessed a strong pneumococcidal action (Experiment 2).

Experiment 2.—Eighteen-hour broth cultures of all four strains were used. Dilutions of optochin hydrochlorid in broth, varying from 1 : 10,000 to 1 : 5,000,000, were freshly made up, tested for sterility, and into tubes containing 5 c.c. of each of these dilutions 0.05 c.c. of each of the above-mentioned cultures was inoculated. The tubes were then incubated at 37 C. and readings made at the end of twenty-four hours and forty hours. Specimens of the eighteen-hour cultures used for incubation were diluted and the dilutions plated in order to be sure that the inoculations were comparable. That they were comparable is shown by Table 23. For convenience the strains have been designated as follows:

Strain A, isolated from the sputum on admission.

Strain B, obtained from the blood on admission.

Strain C, obtained from the left lower lobe immediately after death (spreading lesion).

Strain D, obtained from heart's blood after death.

TABLE 23.—NUMBER OF COLONIES OF PNEUMOCOCCI PRESENT IN 0.00001 C.C. OF EIGHTEEN-HOUR BROTH CULTURES OF STRAINS A, B, C AND D

Dilution, C.c.	Strain A	Strain B	Strain C	Strain D
0.00001	944	2,168	2,228	1,120

Table 23 shows that more or less comparable numbers of bacteria were present in the cultures, of each of which 0.05 c.c. was used for inoculation.

The amount of growth in the tubes (Table 24) was judged macroscopically, and in addition smears from tubes 1 to 5, inclusive, Strain D and from Tube 1, Strain B, showed gram-positive diplococci after forty hours' incubation. From the first three tubes of each strain which showed no growth after forty hours' incubation, 0.5 c.c. was removed and plated in about 20 c.c. of 1 per cent. glucose agar. These plates after forty-eight hours' incubation showed no colonies.

TABLE 24.—Growth of Eighteen-Hour Bouillon Cultures of Strains A, B, C and D in Varying Dilutions of Optochin in Broth at 37 C.

Tubes	Dilution of Optochin in Broth	Strain A		Strain B		Strain C		Strain D	
		22 Hrs.	40 Hrs.	22 Hrs.	40 Hrs.	22 Hrs.	40 Hrs.	22 Hrs.	40 Hrs.
1	1:5,000,000	0	0	0	Heavy	0	0	Heavy	Heavy
2	1:1,000,000	0	0	0	0	0	0	Heavy	Heavy
3	1:750,000	0	0	0	0	0	0	Heavy	Heavy
4	1:500,000	0	0	0	0	0	0	Heavy	Heavy
5	1:250,000	0	0	0	0	0	0	Heavy	Heavy
6	1:100,000	0	0	0	0	0	0	0	0
7	1:50,000	0	0	0	0	0	0	0	0
8	1:10,000	0	0	0	0	0	0	0	0
Control broth without optochin.....		Heavy		Heavy		Heavy		Heavy	

TABLE 25.—Comparison of Growth of Strains A, B, C and D in Normal Human Serum and in Bactericidal Serum Obtained from Case 17 (No. 2638), Designated Serum G

Strain	Serum	Number of Colonies of Pneumococci per 0.5 C.c. Serum When Plated*			
		Immediately	After 2¾ Hours Inc.	After 7½ Hours Inc.	After 21 Hours Inc.
A	Normal	207	822	A. C.	C.
A	G	232	58	7	0
B	Normal	218	796	C.	C.
B	G	984	375	5	0
C	Normal	434	10,000 Est.	C.	C.
C	G	398	43	4	1
D	Normal	730	A. C.	C.	C.
D	G	737	10,000 Est.	C.	C.
Group II	Normal	229	400	C.	C.
Group II	G	263	32	0	0

* Inoculation: 0.00001 to 0.000001 c.c. of eighteen-hour broth cultures.

From Table 24 it is seen that Strains A and B, isolated from the sputum and blood, respectively, on admission and before the patient had received any optochin, and Strain C, isolated from the left lung after death, grew readily in ordinary broth, but were killed in very high dilutions of the drug in broth (1:1,000,000 to 1:5,000,000); on the contrary, Strain D, isolated from the heart's blood immediately after death, grew freely in much greater concentrations of the drug and was only killed in a concentration of 1:100,000 or greater. Furthermore, as shown by Table 25, when incubated in serum obtained from the

patient himself, which showed a definite pneumococcidal action, strains A, B and C and a strain belonging to Group II were readily killed, whereas Strain D was not killed, but grew almost as readily in this serum as in normal human serum.

The facts brought out in these experiments are clear: A strain of pneumococcus isolated from the heart's blood immediately after death was "fast" toward optochin; whereas, strains isolated from the sputum and blood before the administration of optochin and from the lesion in the left lung after death, did not show this condition of fastness. This fastness or resistance to optochin of Strain D was acquired at the most within eight days after the drug treatment was started, and all tests made during this time showed a decided bactericidal action in the patient's blood serum. The actual mode of development of the fastness and the conditions favoring its production are not clear.

In the last twenty-four hours of the patient's life the drug was omitted for a space of seven and one-half hours, after which time it was resumed and a comparatively large dose, 0.5 gm., given at once, followed by two doses of 0.1 gm. It seems unlikely that during this intermission of the drug treatment the concentration of optochin in the blood fell to such a point that fastness was acquired within this time; as we saw in Case 16 (Hosp. No. 2582) (preceding section) that when optochin is given over several days there may be evidence of retention or accumulation of the drug, inasmuch as the serum showed a bactericidal action for as long as ten hours after the last dose.

The possibility exists that a certain degree of fastness was acquired by some of the organisms in the peripheral part of the consolidated area, and that this condition was sufficient to permit them, during the time that the treatment was omitted, to invade the blood stream where they could, during the intermission, and later, conceivably develop a still greater degree of resistance to the drug. It is probable, however, that the fatal outcome of the case is not to be attributed necessarily to the fact that the drug was discontinued for a brief interval. Nevertheless, the fact that the infecting micro-organism was able to become so resistant to the concentration of optochin present in the blood serum during treatment that invasion of the blood stream was not prevented, was probably an important factor in this case in preventing a successful outcome from optochin treatment.

TOXIC EFFECTS

Optochin in too large amounts is toxic for laboratory animals, and in the clinical use of the drug certain toxic symptoms have been observed. So far as known, only one human death has been, in part, attributed to the use of the drug, and in that case it was given intravenously;[10] death was in that case considered to be due to heart failure,

in part caused by the use of optochin; even in this case it is not entirely clear that heart failure was occasioned by the optochin. The toxic symptoms noticed have generally been transient and have usually disappeared when the drug was discontinued. The principal ones that occur are referable to the eye and the ear. In some cases vomiting has been reported; this may be due in some instances to the bitter taste of the drug, which rivals quinin in that respect. Vomiting, however, is rather infrequent, and one disadvantage connected with it is the possibility that the drug may be ejected. In only one of our cases was vomiting frequent enough to interfere with the administration of the drug, and in that case it is doubtful if the optochin was the cause.

The symptoms referable to the ear comprise ringing and roaring noises and partial deafness. In six of our cases tinnitus and partial deafness were associated, and in one case there was tinnitus alone, without deafness. In every case these symptoms were transient, disappearing shortly after the drug was discontinued. Indeed, in some instances these symptoms disappeared or became less evident while the drug was still being given in full amounts.

The symptoms referable to the eyes are more serious. It is difficult to determine the percentage of recorded instances of eye trouble due to the use of the drug; a review of the literature would seem to show that about 4.57 per cent. of all reported treated patients have had transient amblyopia or amaurosis. In one recorded case has there been permanent, almost complete, blindness. In this case, reported by Oliver, the patient received 5 grains (0.3 gm.) every three hours, which would mean 2.4 gm. per twenty-four hours (total amount 7.7 gm.), a dose probably greater than that usually given. In all other cases of amblyopia and amaurosis reported in the literature, the visual disturbances apparently disappeared after the drug had been discontinued. With the impairment of vision the pupils may be widened, may not react to light, and ophthalmoscopic examination may show tortuosity of the retinal vessels, and generally shows narrowing of the arteries. Whether these changes in the pupils and vessels precede the impairment of vision for any considerable time cannot be said at present; they did not appear in any of our cases with the exception of the one that had already developed amaurosis, although all of the patients who received the drug had daily ophthalmoscopic examinations. It is interesting to note that the ophthalmoscopic picture in optochin amblyopia is said to resemble closely that of quinin amblyopia.[27]

27. Since the foregoing was written L. Loránt (Deutsch med. Wchnschr., 1916, **42**, 1355) has described a case of total amaurosis in a woman who apparently received 2.4 gm. of optochin hydrochlorid per twenty-four hours, in doses of 0.2 gm. Vision was only slightly restored six months afterward. Recently, also, we have observed transient amblyopia, lasting one to six hours, in three patients who received optochin hydrochlorid by mouth in amounts sufficient to produce pneumococcidal action in the blood stream. In our cases administration of the drug was discontinued on the first appearance of eye symptoms.

There is some ground for believing that the visual disturbances may depend to a certain extent on idiosyncrasy. That a condition of susceptibility to the drug may be an important factor is indicated by the fact that some patients develop amblyopia after they have had only 2 gm. or less of optochin, whereas others do not acquire it even after they have received as much as from 10 to 16 gm. Study of the literature seems to indicate, however, that the more the daily dosage is subdivided, the less frequent are the visual disturbances. If subsequent observation bears out this point, an additional reason is supplied for not giving the drug in three large doses in twenty-four hours, which method, as shown previously, probably gives rise to considerable fluctuations in the concentration of optochin in the circulating blood. It is possible, also, that the maintenance of the body-weight relationship may help to diminish the frequency of occurrence of this complication by preventing overdosage. Daily examination of the eyegrounds and the pupils may give warning of impending serious symptoms and thus indicate the discontinuance of the drug, or reduction of dosage.

One of the patients included in the present study complained of photophobia during the course of administration of the drug, but on close questioning the patient admitted that this symptom was present before admission to the hospital; the optochin was not discontinued and no impairment of vision resulted. Another patient (Case 18, Hosp. No. 2573) developed an acute retinitis with temporary blindness after receiving four doses of 0.5 gm. each at intervals of eight hours, and deserves to be recorded in detail. For the ophthalmoscopic notes we are indebted to Dr. Otto Schirmer of New York, who followed the case closely and with interest.

Case 18 (No. 2573).—Aged 16, weight 52.6 kg.

Past History.—Unimportant.

Present Illness.—Cough and pain in the chest two days before admission.

Status on Admission: temperature, 103.4 F.; pulse, 120; respirations, 40; involvement of right lower lobe; white blood count, 18,000; blood culture sterile; sputum tenacious, slightly rusty, containing pneumococcus, Group II.

Course: Optochin started on fourth day of disease. All doses were given by mouth. Four doses of 0.5 gm. each were given at eight-hour intervals. After receiving 2 gm. the patient suddenly complained of inability to see. The pupils were found to be dilated and did not react to light or on accommodation. The patient could not distinguish a bright light when flashed directly before her eyes. Ophthalmoscopic examination showed reddish disks, with blurred outlines, gray retinae, tortuous arteries and dilated veins. Diagnosis: acute toxic retinitis (Dr. Schirmer). The administration of optochin was discontinued at this point. The temperature fell by crisis on the eighth day. After six days of total blindness the patient could distinguish light from darkness. Her vision gradually returned, the disks lost their reddish color, the retinae became less hazy, and the arteries became contracted and lost their tortuosity. Three months after the temporary blindness, central vision was 15/20 with each eye; visual fields were contracted to about 10 to 15 degrees and the arteries remained narrow.

Seven months later the visual fields had appreciably increased in extent, and

vision was 15/15 with each eye. The arteries were still contracted; the disks were slightly pale. The patient is working steadily in a factory, and the only abnormal thing she notices is fatigue of the eyes after reading.

The total amount of optochin given was 2 gm. Amount of optochin per kilo of body weight, per twenty-four hours = 0.0285 gm.

The occurrence of amblyopia in 4.57 per cent. of all published cases in which the patients have been treated with optochin constitutes a distinct drawback in the use of the drug. The danger of permanent impairment of vision as a result of the use of optochin should always be borne in mind. Patients undergoing treatment with the drug should be carefully watched and questioned with this possibility in view.

INFLUENCE ON THE CLINICAL COURSE OF THE DISEASE

In attempting to determine the effect of optochin treatment on the course of the disease in the patients reported in this series, the cases will be analyzed from the following points of view: (1) the occurrence, during optochin treatment, of involvement of a hitherto uninfected lobe of the lung, designated as "spread" for brevity's sake; (2) the occurrence of pneumococcal septicemia before or during treatment, or after treatment was discontinued; (3) the duration of the disease, and (4) the mortality rate.

1. *The Occurrence of "Spreads."*—The involvement of a hitherto unaffected lobe during the course of lobar pneumonia is an unfavorable sign. It is an indication that the resistive powers of the individual have not succeeded in arresting the progress of the disease, and it constitutes an additional increment to the burden which the patient is compelled to bear as a result of the infection. In view of these considerations it would seem that the prevention of these so-called "spreads" during the course of specific treatment of lobar pneumonia may be taken as some measure of the ability of the treatment to arrest the progress of the disease.

In seven of the thirty-two patients treated with optochin the pneumonic process spread to an uninvolved lobe during the course of treatment. In judging whether or not such a spread had occurred, we have been guided by the physical signs for the most part, aided, in some instances, by Roentgen-ray or necropsy examinations. It is recognized that involvement of a lobe may take place many hours before definite indication of such is given by the physical signs; this fact has been taken into consideration when deciding whether or not a spread had occurred during treatment. Thus, if the physical signs indicating involvement appeared within less than twenty-four hours after the drug was first given, it was considered that the involvement had actually taken place before treatment had been begun. Some such definite time limit is necessary if the observations are to have any value.

Although seven cases showed such a spread during treatment, an analysis shows that in one instance (Hosp. No. 2466) the patient vomited frequently during the administration of the drug, so that it is impossible to say whether or not she received a sufficient amount of the drug. (No bactericidal tests of the serum were made in this case.) In another case (Case 9, Hosp. No. 2581) the amount of drug given per kilogram of body weight per twenty-four hours was 0.0187 gm., and the bactericidal tests showed that this amount was insufficient to produce bactericidal action at any time in the serum of the patient; consequently, in this instance, it could scarcely be expected that the disease would be arrested by the use of the drug. As a matter of fact, the condition of this patient grew steadily worse for several days while receiving the drug. Another patient (Hosp. No. 2499), weighing 80.4 kg., who received 0.0186 gm. of optochin per kilogram of body weight per twenty-four hours showed evidences of a spread during treatment. Unfortunately no bactericidal tests were carried out in this case, but, from the foregoing studies we should expect that the amount of optochin given was insufficient to produce bactericidal action in the serum. Still another patient (Case 14, Hosp. No. 2593, p. 650), who received an amount insufficient to produce bactericidal action in the serum, as shown by tests, gave definite indications that a spread took place during treatment. Of the seven patients who exhibited a spread of the pneumonic process during administration of optochin, probably three received an amount of the drug which sufficed to produce in the serum a decided bactericidal action on pneumococci.

2. *Pneumococcal Septicemia.*—It would seem that if, under optochin therapy, the blood serum acquires the property of killing pneumococci in the test tube, the presence of pneumococcidal activity in the blood stream should be able to prevent the occurrence of a pneumococcal septicemia or to destroy the micro-organisms already present in the blood stream. Leschke[28] and others saw favorable results from optochin treatment in pneumococcal septicemia.

In six of the cases which are included in this study, positive blood cultures[29] were obtained before the administration of optochin was begun. Of these six, two (Cases 1 and 6, Hosp. Nos. 2600 and 2673) gave negative blood cultures twenty-four hours after the initial dose of optochin, and in each case tests showed the presence of bactericidal action in the patient's serum at that time. Of the remaining four, one (Case 12, Hosp. No. 2711) still showed a positive blood culture twenty-four hours after the initial dose of optochin and at a time when com-

28. Leschke, E.: Deutsch. med. Wchnschr., 1915, **41,** 1359; München. med. Wchnschr., 1914, **61,** 2433; Berl. klin. Wchnschr., 1915, **61,** 634.

29. Ten cubic centimeters of blood were incubated in 150 c.c. of broth as a routine.

plete inhibition, but not bactericidal action, was manifest in the blood serum; one (Hosp. No. 2466) showed a progressive increase in the number of pneumococci in the blood, but vomited frequently during the course of administration of the drug, so that undoubtedly considerable optochin was lost (no bactericidal tests were made); one (Hosp. No. 2508, alcoholic, delirium tremens) died sixteen hours after the initial dose of the drug and before a second blood culture could be made; and one (Case 17, Hosp. No. 2638) showed in the blood immediately after death pneumococci which were "fast" to the concentration of the drug present in the blood stream of the patient during treatment, a concentration which sufficed to kill the strain present in the blood and sputum before treatment was begun. Thus in two of the six cases there is some ground for believing that the optochin may have been instrumental in ridding the circulating blood of living pneumococci.

One patient (Case 14, Hosp. No. 2593) gave a negative blood culture immediately before the optochin was commenced, but shortly before death there were four colonies of *Pneumococcus mucosus* per 1.0 c.c. of blood. During the last thirty hours of life this patient received no optochin, owing to inability to swallow the drug.

These observations do not afford much information concerning the value of optochin treatment on the course of pneumococcal septicemia in lobar pneumonia, except in the one instance in which a strain of pneumococcus was shown to acquire a condition of fastness within the body of the patient.

It is possible that the persistence or occurrence of a pneumococcal septicemia after the patient has received optochin for a period of twenty-four hours does not necessarily indicate that the drug is not able to cope with the septicemia, for in some instances more than twenty-four hours may be necessary for the drug to destroy all the organisms in the circulating blood, as indicated by the bactericidal tests; moreover, the presence of a few pneumococci in the blood stream at any one moment does not necessarily mean that they have been there continuously for a considerable period of time, or that they are actually multiplying in the blood, for it is possible that they may be continually gaining access to the circulating blood from the infected focus, where they may be protected from the action of the optochin in full strength.

3. *Duration of the Disease.*—The determination of the duration of an attack of lobar pneumonia, when crisis does not occur, is not easy; in analyzing the cases from this standpoint it has been necessary to set arbitrary standards as criteria of the termination of the active process. Where the disease did not terminate in a crisis which was unmistakable, it has been considered that the acute process had ended when the rectal temperature reached 100 F., and the patient was obvi-

ously in a satisfactory condition. This method has, of course, disadvantages, but it will serve as a basis for discussion.

The average duration of the disease in twenty-three of the twenty-four cases in which there was recovery was 8.1 days.[30] In twelve of these twenty-three cases optochin treatment was started on or before the third day of the disease and the average duration of the disease in these cases was 7.4 days; the average duration of the disease in the eleven cases in which the optochin treatment was begun after the third day of the disease was 8.9 days. Thus, any considerable shortening of the duration of the disease was not apparent in our cases.

4. *Mortality Rate.*—As a result of the work of Cole[31] and his associates, considerable light has been thrown on the immunologic relationships of the pneumococci found in cases of acute lobar pneumonia. On the basis of certain immunity reactions it has been found possible to divide the pneumococci found in lobar pneumonia into four general groups. In three of these (Groups I, II and III) the members of each group show certain complete cross immunologic reactions among themselves, but not with members of any other group. Group III consists of organisms which, on the basis of cultural and morphologic characteristics, are identical with the type known as *Pneumococcus mucosus.* The individual members of Group IV, thus far, have not been found to show cross immune reactions with one another, or with members of Groups I, II or III.

It has been found that the percentage of cases of lobar pneumonia due to pneumococci of one or other of these groups may vary somewhat from year to year and in different clinics; moreover, the pneumococci belonging to these various groups show quite marked differences, among other things, in their virulence both for human beings and for experimental animals, so that considerable variations are encountered in the mortality rate of cases of lobar pneumonia, depending on the particular group to which the infecting pneumococcus belongs.[32] In view of these facts, a consideration of the mortality rate in any series of cases of lobar pneumonia, treated according to a particular method, is not of full value unless the cases be studied from the standpoint of the groups to which the infecting pneumococci belong. This consideration will be more clearly brought out by a study of Tables 26 and 27 (Cole[33]), which show the average incidence of the various

30. The one patient that received large doses of antipneumococcus serum, Group I, is excluded.

31. Cole, R.: The Archives Int. Med., 1914, **14,** 56. Dochez, A. R., and Gillespie, L. J.: Jour. Am. Med. Assn., 1913, **61,** 727. Avery, O. T.: Jour. Exper. Med., 1915, **22,** 804.

32. Cole, R.: New York Med. Jour., 1915, 1 and 58. Mathers, G.: Jour Infect. Dis., 1915, **17,** 514. Cole, R.: Trans. Cong. Am. Phys. and Surg., 1916, **10,** 138.

33. Cole, R.: Tr. Cong. Am. Phys. and Surg., 1916, **10,** 138.

serologic groups of pneumococci in lobar pneumonia and the mortality rate in each group. The figures have been collected from three hospitals and together they constitute a representative group of cases of this infection.

TABLE 26.—INCIDENCE OF SEROLOGIC GROUPS OF PNEUMOCOCCI IN CASES OF LOBAR PNEUMONIA

Institution	Number of Cases				Per Cent.			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
Hospital of Rockefeller Institute.....	113	104	37	74	34.0	31.0	11.0	22.0
Pennsylvania Hospital.....	22	26	3	34	23.0	27.0	3.0	36.0
Cook County Hospital[34].....	50	25	5	31	45.0	22.5	4.5	28.0
	195	155	45	139	36.0	29.0	8.4	26.0
	Total cases, 534							

TABLE 27.—VARIATIONS IN MORTALITY OF CASES OF LOBAR PNEUMONIA NOT SPECIFICALLY TREATED DEPENDING ON THE SEROLOGIC GROUP OF INFECTING PNEUMOCOCCUS

Institution	Number of Cases								Mortality Percentage			
	Group I		Group II		Group III		Group IV		Group I	Group II	Group III	Group IV
	R*	D*	R	D	R	D	R	D				
Hospital of Rockefeller Inst. ...	82	9	40	15	14	15	58	10	22.0	28.6	52.0	14.7
Pennsylvania Hospital.....	23	9	19	7	1	2	30	4	29.0	27.0	67.0	11.0
Cook County Hospital[34].....	37	13	13	7	1	4	23	5	26.0	28.0	80.0	26.0
Total.....	92	31	77	30	16	21	111	22	25.2	28.0	56.0	14.0
									General Mortality 25 per Cent.			

* R = recovered; D = died.

Table 26, which was based on a study of 534 cases occurring in three clinics, each in a different city, shows that an average of 36 per cent. of all cases of lobar pneumonia due to the pneumococcus are caused by strains of this organism belonging to Group I. This percentage of incidence is greater than that of any of the other three groups; the incidence of Groups II and IV being approximately equal, whereas, infection with strains belonging to Group III is much more infrequent. On the other hand, reference to Table 27 shows that the mortality among cases of infection with Group III is much greater

34. Mathers, G.: Jour. Infect. Dis., 1915, **17**, 514.

than that caused by infection with members of any of the other groups, Group II ranking second in this respect, Group I third and Group IV last. These tables show clearly the importance of determining in every instance the particular group to which the infecting pneumococcus belongs when attempting to draw any conclusions from mortality statistics as to the value of any particular form of treatment in acute lobar pneumonia. Thus, if a particular series of cases studied were due, for the most part, to infection with organisms belonging to Group III, owing to the relatively high death rate (56 per cent.) in this group, the number of expected deaths would be greater than the number of such if the cases were due, for the most part, to organisms belonging to Group IV, in which the death rate is comparatively low (14 per cent.).

Although the number of cases reported in this communication is altogether too small to warrant final conclusions as to the value of the drug in the treatment of lobar pneumonia due to the various immunologic groups of pneumococci, nevertheless, it seems desirable for future reference to record the mortality results from this point of view. In thirty-two cases of lobar pneumonia the patients were treated with optochin; of these, twenty-four recovered and eight died, giving a mortality rate of 25 per cent. Thus, there is apparently no reduction in the death rate. We feel, however, that, in judging of the value of the drug, only those cases should be considered in which the patients were adequately treated, as far as our present knowledge of the subject permits us to decide. Accordingly, only those cases will be considered, from the point of view of mortality rate, in which the patients received sufficient optochin to insure the production of bactericidal action in the blood serum. We must, therefore, exclude from consideration those cases (Hosp. Nos. 2483 and 2573) in which for one reason or another the drug was discontinued shortly after administration had been begun, regardless of the ultimate outcome of the case; those cases (Hosp. Nos. 2581, 2508 and 2499) that failed to receive sufficient optochin per kilo of body weight per twenty-four hours to give rise to bactericidal action in the blood serum during the course of administration of the drug; one case (Hosp. No. 2466), that of a patient with vegetative endocarditis, who vomited frequently during the administration of the drug, so that undoubtedly much of the optochin was lost, and one case (Case 4, Hosp. No. 2659) in which crisis occurred before bactericidal action appeared in the blood. Finally, it was thought proper to exclude one case (Hosp. No. 2711) in which the patient received large doses of the type homologous serum intravenously, in addition to the optochin, and recovered. This case was one due to infection with a strain of pneumococcus belonging to Group I, in which group Cole[35] has obtained

35. Cole, R.: New York Med. Jour., 1915, **101,** 1 and 58; Tr. Cong. Am. Phys. and Surg., 1916, **10,** 138.

very encouraging results from treatment with the type homologous antipneumococcus serum alone, on account of which fact the successful outcome of the case cannot be attributed mainly to the use of optochin. Those cases in which the patients received extract of antipneumococcus serum (Group II or Group III) in addition to the optochin, were not excluded from consideration, because the value of this agent in the treatment of cases of lobar pneumonia has not as yet been established, and also because in each instance the extract was not given until after the drug had been administered over a sufficiently long period and in sufficient amounts to produce bactericidal action in the patient's serum. Moreover, antiserums to Groups II and III are by no means as potent in protective power as the antiserum to Group I, and the Group II antiserum, which is more potent than that of Group III, has thus far proved disappointing in clinical practice. The pneumococcidal action of the serum in vitro was shown not to have been enhanced by the administration of the serum extract.

In all, eight cases have been excluded; of these, four patients recovered and four died, one of the latter (Hosp. No. 2508, alcoholic, delirium tremens) in sixteen hours after the first dose of optochin. According to the revised statistics, twenty-four patients were considered to have been adequately treated with optochin, and of these, twenty recovered and four died, making a mortality rate of 17 per cent. The incidence of the various groups in this series of cases and the mortality rate of each group are represented in Tables 28 and 29, respectively.

TABLE 28.—Incidence of Serologic Groups of Pneumococci in Cases of Lobar Pneumonia Patients Treated with Optochin

Group	Number of Cases	Per Cent.
II	10	42
III	7	29
IV	6	25
Unclassified	1	4
Total	24	

Table 28 shows that of the patients that may be legitimately regarded as having been adequately treated with optochin, the majority of cases were caused by organisms belonging to Groups II and III. When it is recalled that, according to Table 27, the mortality among the patients whose cases were due to pneumococci belonging to these groups is greater than that among cases due to pneumococci of Groups

TABLE 29.—MORTALITY IN CASES OF LOBAR PNEUMONIA PATIENTS TREATED WITH OPTOCHIN, ARRANGED ACCORDING TO SEROLOGIC GROUPS

Group	Number of Cases	Recovered	Died	Mortality Rate, Per Cent.
II	10	9	1	10
III	7	5	2	29
IV	6	5	1	17
Unclassified	1	1	0	0
Total	24	20	4	17

I and IV, it will be seen that, although this series of cases is small, it nevertheless represents the severest types of infection with which one has to deal in cases of lobar pneumonia; therefore the drug may be said not to have had an easy trial. As explained earlier, only two patients belonging to Group I were treated with optochin. Table 29 shows the actual mortality rate in the various groups. Table 30 contrasts the actual mortality rate with the expected mortality rate in twenty-three treated patients, based on the figures given in Table 27, and also the number of deaths expected with that actually encountered (Hosp. No. 2672, unclassified, recovered, is excluded). A reduction in the mortality rate over that expected was observed in cases due to Groups II and III, and no reduction in cases due to Group IV. The number of cases due to strains belonging to Group I was too small to warrant recording, in this estimation, the results of the treatment in cases falling in this group. The total expected mortality rate, therefore, in this series of twenty-three cases was 33 per cent., and the total actual mortality rate was 19 per cent.

TABLE 30.—CONTRAST BETWEEN EXPECTED AND ACTUAL MORTALITY IN TWENTY-THREE PATIENTS TREATED WITH OPTOCHIN

Group	Number of Cases	Expected Mortality Rate, per Cent.	Actual Mortality Rate, per Cent.	Number of Expected Deaths	Number of Actual Deaths
II	10	28	10	3	1
III	7	56	29	4	2
IV	6	14	17	1	1
Totals	23	33	19	8	4

A summary of the complete series of cases is given in Table 32.

DISCUSSION

The aim of specific chemotherapy in lobar pneumonia should be to destroy the pneumococci in the affected portions of the lung, to confine the pathologic process to the part of the lung involved, and to prevent the occurrence of empyema and of septicemia, as well as complications resulting therefrom; in short, to prevent direct or indirect extension of the morbid process from the focus of disease.

It is obvious that if a drug is to be used as a specific in the treatment of this disease it must possess a bactericidal action on pneumococci in the presence of body fluids; further, if it is to be given by mouth, it must be capable of being absorbed from the gastro-intestinal tract into the blood stream, and of being there maintained for an adequate period at a concentration sufficient to kill pneumococci without causing harm to the patient at the same time. To be of maximum value in the control of the disease, it should possess the property of passing through the capillary walls into the alveolar spaces and of penetrating the inflammatory exudate there encountered.

Optochin hydrochlorid fulfils at least some of these requirements under certain definite conditions. First, the drug, even in great dilution, possesses a marked bactericidal action on pneumococci in vitro in the presence of human blood serum and whole blood. In the second place, insofar as may be judged from the present study, which comprises too few cases to warrant final conclusions, it is capable of being absorbed from the gastro-intestinal tract into the blood stream in such amounts that a certain degree of pneumococcidal power may be maintained there for several days, provided the following conditions are fulfilled:

1. That the amount of the drug given per kilogram of body weight per twenty-four hours be at least 0.024 gm. (Amounts smaller than this, as low as 0.02 gm., may be sufficient to produce bactericidal action in the serum, but they cannot always be relied on to do so.)

2. That the size and spacing of the individual doses be suitably arranged.

Under such a regulation of dosage, however, amblyopia may result. This complication has occurred in 4.57 per cent. of all cases reported in the literature, and was generally recovered from; its occurrence in the future may possibly be lessened when the drug is given in accordance with a method such as that outlined above; there seems to be no doubt that some of the cases in which visual disturbances occurred received a larger amount of the drug than that necessary to insure the production of bactericidal action in the serum.

Whether or not the drug can pass through the capillaries of the pulmonary vessels and penetrate the fibrinous exudate in the alveolar

spaces cannot be stated. That it may pass through the capillary wall into a serous sac is demonstrated by one of our cases (Case 2, Hosp. No. 2705) which received optochin by mouth for several days, and in which it was possible to demonstrate in the pericardial fluid, obtained immediately after death, a bactericidal action on pneumococci in vitro as great in degree as that present in the blood serum shortly before death.

The available experimental evidence, therefore, apparently goes to show that optochin fufils at least some of the requirements for its use as a specific chemotherapeutic agent in the treatment of acute lobar pneumonia, its chief drawback being the possibility that it may exert, in some instances, a toxic action on the eye. The ultimate decision as to the value of the drug in this disease, however, must rest on clinical experience based on its use in a large series of cases. In order to arrive at a scientific evaluation of its utility, it should be used systematically and according to a method that will insure the rapid production of bactericidal action in the serum and the maintenance of the same at a more or less constant level, within the limits of safety for the patient.

The rapid production and maintenance of the bactericidal action in the blood seem desirable in order that the infecting pneumococci may not acquire a condition of "fastness" to the drug, by virtue of which they become insusceptible to its action. That this consideration is not wholly theoretical is evidenced by the fact that pneumococci can acquire this property in vitro, and, further, by the demonstration that, in one instance (Case 17, Hosp. No. 2638), a strain of pneumococcus recovered from the heart's blood immediately after death was fast not only to concentrations of optochin much greater than that necessary to kill the strain obtained from the patient's blood on admission, but also to that concentration of optochin present in the blood of the same patient during the height of the treatment.

As already stated, the fact that the severity and mortality of the disease bear some relation to the particular group to which the infecting pneumococcus belongs, must be taken into account in an attempt to estimate the value of any treatment of lobar pneumonia. It should be emphasized that Groups II and III are the most virulent of the groups thus far recognized, and that the death rate in patients infected with strains belonging to these groups is greater than in the case of the other groups. Inasmuch as the majority of the cases treated in this study were due to infection with members of these two groups, the drug may be said to have had a severe test. In the revised figures, after exclusion of Case 12 (Hosp. No. 2711, recovered), in which other efficient therapeutic measures were employed, of one unclassified case (Hosp. No. 2672, recovered), and of seven cases (of which three

recovered and four died) where the optochin treatment for one reason or another was, on the basis of experimental or clinical observations, almost certainly inadequate, the expected mortality among twenty-three cases was calculated to be 33 per cent., whereas the actual mortality was found to be 19 per cent. It must, however, be again emphasized that the number of cases in the series is far too few to permit of final conclusions.

In this paper stress has been laid on the rapid attainment and maintenance in the circulating blood of a concentration of optochin sufficient to exert pneumococcidal action; further, it has been urged that the dosage of the drug should be regulated with this bactericidal action as a criterion, since this is the only objective method available for determining the optimum system of dosage. It must be borne in mind that a concentration of optochin in the circulating blood sufficient to exert bactericidal action in vitro may not, necessarily, be sufficient to influence favorably the course of the disease in the human body, for several reasons. It is probable that, for the drug to influence the course of lobar pneumonia to the greatest extent, it must penetrate the inflammatory exudate in the alveolar spaces and bring about the destruction of the pneumococci there present. To bring about the passage of the drug through the capillary walls into the exudate, a concentration of optochin in the blood stream in excess of that required to produce bactericidal action in the serum may be necessary. Whether or not this is the case cannot be stated on the basis of our present knowledge of the conditions governing the behavior of the drug in the human body; it is certain that the toxicity of the drug sets a limit to the concentration of optochin which may be attained in the serum in any given case. In one instance in the present study, as already stated, the drug was shown to be present in the pericardial fluid after oral administration. If this observation[26] should be confirmed in the future, the evidence will be in favor of the view that the drug can, ordinarily, make its way through the capillary wall into the alveolar spaces, although not necessarily into the exudate. Here it may be noted that empyema did not occur in any case of the present series.

The possibility of relative impermeability for optochin of the inflammatory exudate in the alveolar spaces suggests that the early use of the drug, before consolidation is complete, may be of importance, and that such a condition of comparative impermeability, occurring later in the disease, after complete consolidation has taken place, may be the reason why, according to several writers, better results are obtained in patients treated on or before the third day of the disease. In two instances in the present series, where treatment was started not later than the second day, rather striking results were obtained clinically, although the cases were due to infection with pneumococci belonging

to Group III, which shows a mortality greater than 50 per cent., and in which the disease generally runs a more severe course than in the case of infection with strains of the other groups.

Even if the drug is not capable of destroying all the pneumococci in the infected area when present in the blood in a concentration sufficient to produce bactericidal action in the serum in vitro, nevertheless, if it can prevent a spread of the disease process to uninfected areas and prevent the establishment of septicemia or metastatic complications, its use may be helpful; for experience seems to indicate that the occurrence of involvement of hitherto uninfected areas constitutes a source of danger to the patient.

The simultaneous use of optochin and antipneumococcus serum in the specific treatment of lobar pneumonia would seem to offer considerable hope. Until recently the treatment of lobar pneumonia with specific antiserums has not been attended with striking results; this has probably been due, in part, to the failure to recognize the extent and significance of the immunologic differences existing among the pneumococci. Through the recognition of these differences it has become apparent that, in order to achieve any positive results in the treatment of lobar pneumonia with antiserums, a serum corresponding to the particular group to which the infecting pneumococcus belongs must be employed. Recently Cole[32] has reported encouraging results obtained in a series of cases of lobar pneumonia due to infection with strains belonging to one of the immunologic groups (Group I) through the use of intravenous injections of the corresponding antiserum. In seventy-two consecutive patients thus treated the mortality was 8.3 per cent., whereas the death rate among untreated patients in this group is 25 per cent., so that specific serum therapy has undoubtedly succeeded in considerably lowering the death rate in at least one group of cases of lobar pneumonia. The use of antipneumococcus serum in Group II has, by itself, proved disappointing up to the present in the treatment of lobar pneumonia. Antipneumococcus serum has definite specific protective power against infection with a strain of the corresponding group in mice, and one of us (M.),[36] has shown that the protective value of the antiserum to Group II may be increased considerably if the animals receive a small dose of optochin simultaneously with the serum. This fact would seem to suggest that, in the specific treatment of lobar pneumonia, perhaps even more favorable results may be expected from the simultaneous employment of optochin and the type homologous antipneumococcus serum, than by the use of either of these remedies separately.

On the basis of the evidence presented in this communication the

36. Moore, Henry F.: Jour. Exper. Med., 1915, **22**, 389.

following suggestions are made for the use of the drug, although the observations are too few in number to permit of dogmatic statements.

1. It would seem, as far as present knowledge goes, that optochin hydrochlorid is best administered by the mouth in acute lobar pneumonia. (The drug is preferably given in capsules, because of its bitter taste.)

2. The drug should be administered in such amounts as will insure the rapid production and more or less constant maintenance, within the blood stream, of a concentration sufficient to exert bactericidal action on the pneumococci.

3. To achieve this result, the total amount of optochin given by the mouth per twenty-four hours should bear a definite relationship to the body weight of the patient, namely, at least 0.024 gm. of optochin hydrochlorid per kilogram of body weight. (It is possible that, after one or more days' administration of the drug, lesser amounts may suffice owing to retention or accumulation within the body.)

4. To secure the rapid production of bactericidal action in the blood stream the initial dose should be larger than the subsequent ones, the body weight relationship being at all times preserved.

5. The individual doses should be given regularly throughout the treatment and the intervals between the doses should, in general, not exceed two to three hours. Thus, in the case of individuals of average weight, say of 62 kg., 0.024 gm. per kilogram of body weight per twenty-four hours would mean a total of 1.5 gm. of the drug per twenty-four hours (Table 31).

TABLE 31.—SCHEME OF DOSAGE FOR INDIVIDUAL OF AVERAGE WEIGHT (62 KG.)

First 24 hours of treatment	Initial dose of 0.45 gm. Interval three hours Seven doses of 0.15 gm. each, with an interval of three hours between each two doses	Total, 1.5 gm.
Subsequent periods of 24 hours each	Ten doses of 0.15 gm. each, with an interval of about 2½ hours between each two doses	Total, 1.5 gm.

When the patient is below average size, the utilization of the same body weight relationship should insure the production of the bactericidal action in the blood stream, as shown above (Cases 10, Hosp. No. 2675, and 11, Hosp. No. 2626, p. 644); such patients require a smaller total quantity of optochin per twenty-four hours than patients of greater weight. For example, a patient weighing 42 kg. should require only 1.0 gm. of optochin per twenty-four hours. The size and spacing of the individual doses in such a case is indicated by the treatment of Cases 10 and 11 (Hosp. Nos. 2675 and 2626, Tables 13 and 14, pp. 644 and 645).

When the patient is above average weight, in order to preserve the

desired relationship between the amount of the drug and the body weight, more than 1.5 gm. must be given per twenty-four hours. Thus, a patient of 80 kg. would receive a total quantity of 1.9 gm. per twenty-four hours. Whether this comparatively large dose is safe or not, we cannot say. The possibility of untoward effects should always be remembered not only when such larger total daily doses are used in larger patients, but also in the case of small or average sized persons, even though the body-weight relationship be maintained. In the use of the drug impairment of vision should be carefully looked for and the drug should be discontinued, or at least reduced in amount, on its appearance. Daily ophthalmoscopic examinations might be advantageous.

Experiments in vitro have shown that the pneumococcidal action of optochin is manifest in very high dilution, and that the concentration required for the production of bactericidal action is not much greater than that which produces inhibition of growth. If an average sized individual, say of 60 kg., receive 1.5 gm. of optochin, and a large individual, say of 80 kg., receive the same dose per twenty-four hours, the former would get 0.007 gm. of the drug per kilogram of body weight per twenty-four hours more than the latter, an amount which we should, from our experience of the drug in vitro, expect to be sufficient to replace mere inhibition by actual bactericidal action in the serum.

The present study comprises too few cases to permit of final decision regarding the optimum dosage of the drug or its value in the treatment of lobar pneumonia; these points can only be ascertained when a much larger series of cases is available for analysis.

CONCLUSIONS

1. Variations in the concentration of ethylhydrocuprein (optochin) hydrochlorid in normal horse serum are evidenced by corresponding variations in the degree of inhibitory and bactericidal power on pneumococci, when the drug is allowed to act on these micro-organisms in the test tube at 37 C.; the degree of the specific inhibitory or bactericidal power of optochin-containing serum gives an indication of the concentration of optochin present.

2. The members of a four-hour bouillon culture of pneumococcus are more resistant to the action of optochin than those of a twenty-hour culture (37 C.).

3. The use of "young" (four to six hours at 37 C.), actively growing bouillon cultures of pneumococci in bactericidal tests, affords a more delicate index of the differences in the degree of inhibitory or bactericidal action of different concentrations of optochin than does the use of older (twenty hours at 37 C.) cultures.

TABLE 32.—

Case No.	Hosp. No.	Age	Weight, Kg.	Group of Infecting Pneumococcus	Blood Culture Before Treatment	Day of Disease When Optochin Treatment Was Begun	Method of Dosage of Optochin in Periods of 24 Hours	Amount of Optochin, in Gm. per Kilo of Body Weight per 24 Hours (First Two Periods)	Total Amount of Optochin in Gm.	Duration of Treatment with Optochin, Days (24 Hrs.)	Duration of Disease, Days
	2477	26	52	II	Sterile	4	3 × 0.5	0.0288	4.5	2.75	7
	2496	22	67.4	II	Sterile	3	3 × 0.5	0.0222	6.0	4	5
	2521	34	57.4	II	Sterile	7	10 × 0.15	0.0261	7.5	5	12
	2482	40	81.6	II	Sterile	3	3 × 0.5	0.0183	3.0	2	8
9	2581	35	80	II	Sterile	3	1 × 0.5 + 7 × 0.15; 10 × 0.15 thereafter	0.0187	3.8	2.5	11
18	2573	16	52.6	II	Sterile	3	2 × 0.5	0.0285	2	1.3	9
7	2611	60	58.4	II	Sterile	2	1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0256	5.7	3.75	7
	2586	26	65.4	II	Sterile	3	1 × 0.45 + 7 × 0.15; 10 × 0.15	0.0229	5.25	3.5	7
	2486	16	44.9	II	Positive; 40 col. per 1.0 c.c.	2	2 × 0.5; 6 × 0.25; vomited frequently	?	?	2.0	4
11	2626	15	42.2	II	Sterile	6	1 × 0.2 + 9 × 0.1; 12 × 0.1; 8 × 0.15 thereafter	0.0284	5.65	4.6	11
6	2673	39	81.6	II	Positive	3	1 × 0.5 + 9 × 0.15; 13 × 0.15; 2(12 × 0.15); 11 × 0.15	0.0226 0.0238	9.5	5.5	8
	2502	65	62	II a	Sterile	3	10 × 0.15	0.0242	6.6	4	7
15	2604	20	55.4	II a	Sterile	4	1 × 0.15 intramus. + 7 × 0.15 by mouth; 10 × 0.15 thereafter by mouth	0.0270	4.5	3	7
13	2658	29	66.6	II a	Sterile	2	1 × 0.5 intramus. + 4 × 0.25 intramus.; 10 × 0.15 thereafter by mouth	0.0225	2.9	2.5	5
	2508	48	75 est.	III	Positive; 2 col. per 1.0 c.c.	4	At rate of 10 × 0.15	0.020 est.	1.2	0.75	5
8	2566	21	67.2	III	Sterile	2	1 × 0.45 + 7 × 0.15; 10 × 0.15	0.0223	2.9	2.75	6
	2550	62	72.3	III	Sterile	2	1 × 0.5 + 7 × 0.15; at rate of 10 × 0.15 thereafter	0.022	2.6	8	11
16	2582	48	45.2	III	Sterile	3	1 × 0.4 + 6 × 0.15; 2 (10 × 0.15)	0.0286 0.063	8.5	8	11

—SUMMARY OF CASES

Latest Time of Appearance of Bactericidal Action in Serum of Patients after Initial Dose of Optochin	Toxic Symptoms Referable to Optochin	Complications	Result	Occurrence of "Spread" During Treatment	Remarks
Not studied	None	None	Recovered	None	Physical signs persisted one week after temperature became normal
24 to 34 hours	Necrosis at site of subcutaneous injection	Serous pleurisy	Recovered	None	Extremely ill patient; temperature remained elevated several days owing to serous pleurisy; second dose of optochin vomited; fourth and sixth doses given subcutaneously; fifth given intramuscularly
8 to 23 hours	None	None	Recovered	None	Alcoholic; constant delirium
No bactericidal action	None	Pericarditis	Died, 8th day of disease	None	Drug was discontinued three days before death
No bactericidal action	None	None	Recovered	Spread	Optochin discontinued and "extract" of antipneumococcus serum (Group II) given subcutaneously; no apparent effect of optochin on disease; total "extract" = 103 c.c. = 583 c.c. whole serum
5.25 hours	Retinitis with temporary blindness	None	Recovered	None	Drug discontinued after onset of amaurosis; eighteen-hour culture used for tests
15 hours	Temporary deafness and tinnitus	None	Recovered	None	Delayed resolution; extract of antipneumococcus serum (Group II) subcutaneously, commenced 48 hours after optochin was started; total "extract" = 40 c.c. = 220 c.c. whole serum
37.5 hours, "young" cult. (very heavy seeding); "old" cult., 9 hours	None	None	Recovered	None	"Extract" of antipneumococcus serum (Group II) subcutaneously on fifth, sixth and seventh days; total "extract" = 47 c.c. = 235 c.c. whole serum
Not studied	Nausea and vomiting	Endocarditis	Died, 4th day of disease	Spread	Progressive septicemia
17.5 to 24.5 hours	Temporary deafness	None	Recovered	None	Chronic otitis media on admission
12 hours	None	None	Recovered	Spread	Blood culture positive before treatment; sterile 24 hours after first dose of optochin; extract of antipneumococcus serum (Group II) intravenously, commenced 36 hours after optochin was started; total "extract" = 155 c.c. = 620 c.c. whole serum
Not studied	None	None	Died, 7th day of disease	None	
42 hours	None	None	Recovered	None	Extract of antipneumococcus serum (Group II) started intravenously and subcutaneously 48 hours after first dose of optochin; total "extract" = 22 c.c. = 111 c.c. whole serum
18 hours	None	None	Recovered	None	Bactericidal action appeared at 19½ hours, then disappeared and reappeared at 28.5 hours
No bactericidal action in 17 hours	None	None	Died 17 hrs. after treatment began	None	Alcoholic; delirium tremens; restraint necessary
12 hours	None	None	Recovered	None	Bactericidal action disappeared when dosage was decreased
2.75 hours	Temporary tinnitus	None	Recovered	None	Physical signs persisted 2 days after temperature became normal; eighteen-hour old culture used in tests
1.75 hours	Temporary deafness	None	Recovered	None	Slow defervescence; eighteen-hour old culture used in tests; drug omitted for 13½ hours on fourth day of treatment

TABLE 32.—

Case No.	Hosp. No.	Age	Weight, Kg.	Group of Infecting Pneumococcus	Blood Culture Before Treatment	Day of Disease When Optochin Treatment Was Begun	Method of Dosage of Optochin in Periods of 24 Hours	Amount of Optochin, in Gm. per Kilo of Body Weight per 24 Hours (First Two Periods)	Total Amount of Optochin in Gm.	Duration of Treatment with Optochin, Days	Duration of Disease, Days
14	2593	37	53.8	III	Sterile	2	1 × 0.4 intramus. + 7 × 0.15 by mouth; 6 × 0.15 by mouth; 9 × 0.15 by mouth; 8 × 0.15 by mouth; 10 × 0.15 by mouth;	0.0269 0.0167 (0.0250) (0.0223) (0.0276)	7.9	6	7
5	2540	46	55.8	III	Sterile	4	1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0268	9.45	7	10
	2589	58	61	III	Sterile	2	1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0270 0.0245	7.05	5.5	8
2	2706	34	82.8	III	Sterile	2	3 × 0.45; 1 × 0.45 + 7 × 0.15; 2 (9 × 0.15); 10 × 0.15 thereafter	0.0255 0.0284	9.9	7	8
	2409	58	80.4	III	Sterile	4	10 × 0.15	0.0186	6.0	4	8
	2588	55	74.4	IV	Sterile	4	1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0201	4.5	4	8
1	2600	34	65.4	IV	Positive	5	3 × 0.5; 1 × 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0229	3.6	2.5	8
	2624	42	70.3	IV	Sterile	6	1 + 0.45 + 7 × 0.15; 10 × 0.15 thereafter	0.0213	5.25	3.5	11
3	2629	41	72.8	IV	Sterile	2	11 × 0.15	0.0226	4.05	2.5	5
10	2675	15	39.1	IV	Sterile	4	1 × 0.3 + 7 × 0.1	0.0253	1.0	1	5
17	2688	55	42.3	IV	Positive	4	1 × 0.5 + 1 + 0.15 + 7 × 0.1; 3 (12 × 0.1); 7 × 0.1 + 2 × 0.15; 9 × 0.1, etc.	0.0315 0.029	8.75	8	12
4	2650	29	74.5	I	Sterile	6	12 × 0.15	0.0241	1.8	1	7
12	2711	21	57.4	I	Positive	4	1 × 0.45 + 8 × 0.15; 10 × 0.15 thereafter	0.0287 0.026	4.05	2.5	7
	2672	54	66	?	Sterile	6	1 × 0.45 + 7 × 0.15; 10 × 0.15	0.0227	7.8	5	12

—SUMMARY OF CASES

Latest Time of First Appearance of Bactericidal Action in Serum of Patients after Initial Dose of Optochin	Toxic Symptoms Referable to Optochin	Complications	Result	Occurrence of "Spread" During Treatment	Remarks
No bactericidal action in 68.75 hours	Deafness; tinnitus	None	Died, 9th day of disease	Spread	Apparently no effect on course of disease; blood culture positive before death; optochin stopped 30 hours before death
5.5 hours	None	None	Recovered	None	Consolidated lung resolved slowly
26 hours	Tinnitus; slight deafness; both temporary	None	Recovered	None	Eight hours elapsed between first and second doses of optochin; Extract of antipneumococcus serum (Group III) subcutaneously; first dose 69 hours after optochin was started; total "extract" = 20 c.c. = 200 c.c. whole serum
11 hours	None	Serous pericarditis	Died, 8th day of disease	Spread	Extract of antipneumococcus serum (Group III) intravenously after optochin had been administered for six days; total "extract" = 55 c.c. = 550 c.c. whole serum
Not studied	None	None	Died, 8th day	Spread	
68 to 92 hours	None	None	Recovered	None	After 24 hours of administration of optochin the drug was omitted for 20 hours and then resumed
11 hours	None	None	Recovered	None	Blood culture positive before treatment; sterile 24 hours after first dose of optochin
26 hours	Tinnitus; slight deafness; both temporary	None	Recovered	None	
16.5 hours	None	None	Recovered	None	
15.5 hours	None	None	Recovered	None	
11 hours	None	None	Died, 12th	Spread	Strain "fast" to optochin was recovered from blood, postmortem
18 hours	None	None	Recovered	None	Crisis 8 hours after commencement of optochin
2 to 4 hours	None	None	Recovered	None	Antipneumococcus serum (Group I) intravenously; first injection 24 hours after first dose of optochin; blood culture positive before and after 24-hour treatment with optochin; total serum given = 300 c.c.
24.5 hours after 1st dose; present first specimen	None	None	Recovered	None	

4. As judged from the present series of thirty-two cases, when optochin hydrochlorid is given by mouth to patients suffering from acute lobar pneumonia in such amounts that they receive at least 0.024 gm. per kilogram of body weight per twenty-four hours, and when the size and spacing of the individual doses are adequately regulated (see Dosage C, p. 627), a specific pneumococcidal action appears in their blood within a few hours, and it can be maintained more or less constant for several days.

5. In order to maintain the bactericidal action in the blood at a constant level the intervals between the individual doses given by mouth should not ordinarily exceed about two and one-half to three hours (see Dosage C, p. 627).

6. When optochin is given by mouth according to such a scheme of dosage as outlined, the evidence points to some retention or accumulation in the blood of part of the drug absorbed.

7. Administration of optochin hydrochlorid by mouth appears to be more satisfactory than intramuscular administration. Further study of intramuscular administration appears to be desirable.

8. Pneumococci, not only in vitro, but also in the human body in patients treated with optochin, may acquire the property of more or less complete resistance or "fastness" to the drug.

9. Toxic symptoms, such as tinnitus, deafness, amblyopia or amaurosis (retinitis) may be observed in the use of the drug in man; they are generally transient. Retinitis, however, may result in more or less permanent impairment of vision.